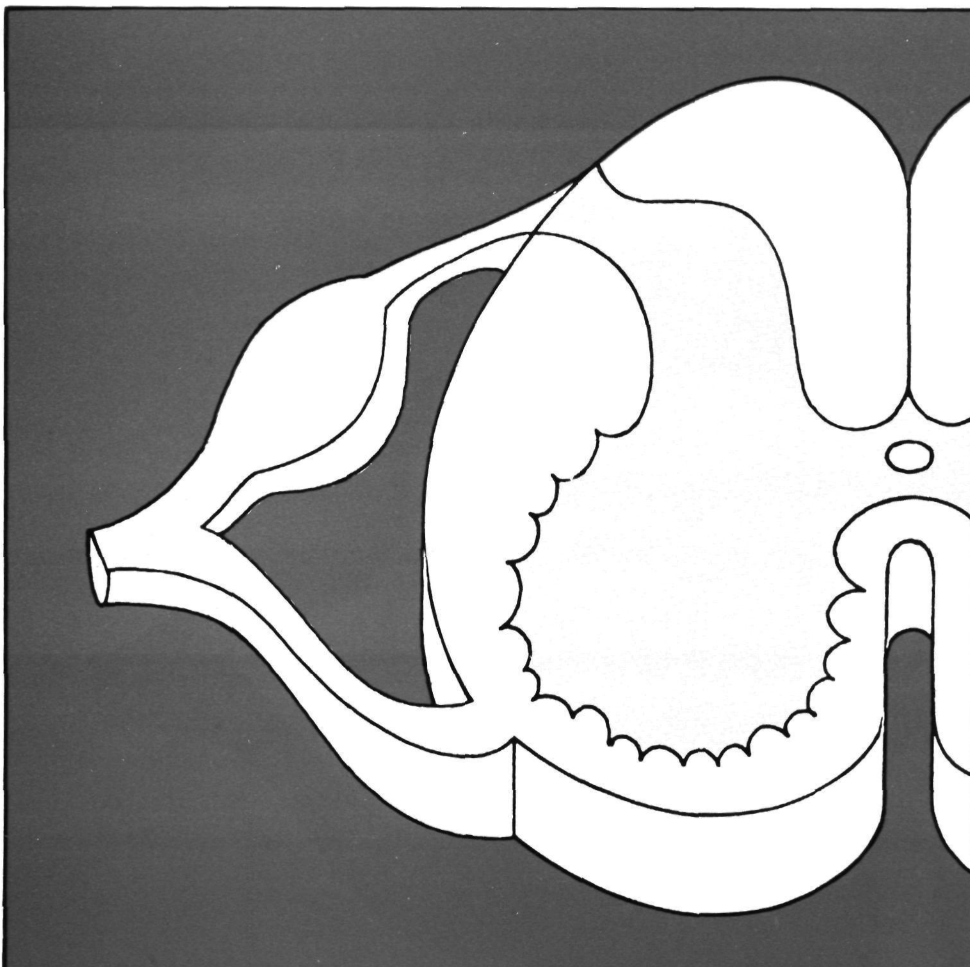
THE ORGANIZATION OF THE SPINAL CORD IN REPTILES WITH DIFFERENT LOCOMOTOR PATTERNS

A. KUSUMA



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PROMOTOR: PROF. DR. R. NIEUWENHUYS

CO-REFERENT: DR. H.J. TEN DONKELAAR

The investigations were supported in part by a grant from the Foundation for Medical Research FUNGO which is subsidized by the Netherlands Organization for the Advancement of Pure Research Z.W.O.

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PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
IN DE GENEESKUNDE
AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. P.G.A.B. WIJDEVELD,
VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN
IN HET OPENBAAR TE VERDEDIGEN
OP DONDERDAG 17 MEI 1979,
DES NAMIDDAGS TE 2 UUR PRECIES

DOOR

ARINARDI KUSUMA

GEBOREN TE MALANG (INDONESIË)

1979

Stichting Studentenpers Nijmegen

ACKNOWLEDGEMENTS

The present thesis was carried out at the Department of Anatomy and Embryology (Head: Prof. Dr. H.J. Lammers), University of Nijmegen, Faculty of Medicine, Nijmegen, the Netherlands.

The author wishes to express his gratitude to Miss Annelies Pellegrino, Mrs. Carla de Vocht - Poort and Miss Nellie Verijdt for the preparation of the histological material, to Mr. Hendrik Jan Janssen and Mrs. Roelie de Boer - van Huizen for expert technical assistance, to Mr. Joep de Bekker and Mr. Joop Russon for the drawing, and to Mr. A.Th.A. Reijnen and Miss Annelies Pellegrino for the photomicrographs.

Grateful appreciation is expressed to Mrs. Trudy van Son - Verstraeten for typing the manuscript.

Ter nagedachtenis aan mijn vader

aan mijn moeder

voor Irene

Part of the present thesis has been published in the series 'Biology of the Reptilia' (C. Gans, ed.), Volume 10, Academic Press, London, 1979, in press.

CONTENTS

I INTRODUCTION

Reptiles are a particularly interesting object for neurobiological research, since their great variation in form and locomotion has remarkable repercussions in the central nervous system. The profound differences with regard to shape and development of the trunk, tail and extremities are clearly reflected in the macroscopic structure of the spinal cord.

Experimental studies in various reptiles using different types of progression, have indicated that the distribution of dorsal root fibers into the spinal cord (Joseph and Whitlock, 1968a) as well as the organization of descending pathways to the spinal cord (ten Donkelaar, 1976a, b) is definitely related to the type of locomotion used.

The aim of the present study is to analyse the cell and fiber pattern of the spinal cord in some reptiles using highly different types of locomotion. With regard to their mode of progression, reptiles can be roughly divided into the following three groups: a) those using for their locomotion solely trunk musculature: limbless lizards and snakes; b) reptiles, moving by way of trunk musculature as well as by way of their extremities: lizards and crocodiles; and c) a group employing only their extremities: chameleons and turtles.

For the present inquiry of each of these three groups one representative was chosen, viz., the snake *Python reticulatus* (Suborder: Ophidia, Order: Squamata), the lizard *Tupinambis nigropunctatus* (Suborder: Sauria, Order: Squamata), and the turtle *Testudo hermanni* (Order: Testudines). In addition, the turtles *Pseudemys scripta elegans* and *Pelomedusa subrufa*, and the lizard *Varanus exanthematicus* have been used.

The present thesis comprises a systematic comparative analysis of the cytoarchitecture and the fiber pattern based on normal material as well as an experimental analysis of the fiber connections of the spinal cord.

Before presenting the above-mentioned topics some notes on gross morphology (Chapter III) will be given, and the general relations of the gray and white matter as well as the characteristics of the various regions of the spinal cord (Chapter IV) will be discussed.

As a preliminary step to the experimental analysis of the fiber connections of the spinal cord it was felt necessary to start the present study with a systematic comparative analysis of the cytoarchitecture (Chapter V) and the fiber pattern (Chapter VI) based on normal material. The material includes transversely sectioned series of the spinal cords of the three species mentioned above. For the analysis of the cell pattern, one or more series of each of the three species studied were stained with cresylechtviolet. The fibers were studied in series stained according to Häggqvist's (1936) modification of the Alzheimer Mann methylblue-eosin stain. This technique stains the axons blue and the myelin sheath of the individual fibers red. In Häggqvist material many bundles and tracts can be clearly distinguished from their environment on account of their characteristic fiber pattern (cf. e.g. van Beusekom, 1955; van den Akker, 1970; Verhaart, 1970; ten Donkelaar and Nieuwenhuys, 1979). The descending fiber systems to the cord, for example, stand out conspicuously in the brain stem as well as in the spinal cord because of their high contingent of coarse fibers. In addition, material stained according to Klüver and Barrera (1953) has been used.

As regards the cell pattern, so far a systematic comparative analysis of the reptilian spinal gray has not been carried out, although some remarks upon this subject can be found in Ariëns Kappers, Huber and Crosby (1936) and in a review on comparative aspects of the spinal cord by Nieuwenhuys (1964). Recently, in the lizard *Tupinambis nigropunctatus*, a subdivision of the spinal gray into layers comparable to those in the cat (Rexed, 1952, 1954, 1964) has been reported (Cruce, 1975, 1979).

The second part of the present thesis addresses itself to an experimental analysis of the fiber connections of the spinal cord. The following fiber contingents will pass in review: dorsal root projections (Chapter VII), propriospinal connections (Chapter VIII), ascending projections from the spinal cord to the brain stem and descending pathways to the cord (Chapter IX).

Our knowledge concerning the fiber connections of the reptilian spinal cord is still very incomplete. Dorsal root transections have been carried out at some levels of the spinal cord in various reptiles (Goldby and Robinson, 1962; Joseph and Whitlock, 1968a, b; van der Sloot, 1968; Cruce, 1979). Apart from determining the termination of the dorsal root fibers in the spinal cord it has been demonstrated that a certain portion of the primary afferent fibers of the dorsal root enters the funiculus dorsalis, and thence passes rostrally to reach the brain stem. These long ascending fibers, terminating in the dorsal funicular nuclei, are somatotopically arranged in such a fashion that fibers of caudal origin are most medial and those adjoining at more rostral levels are situated more laterally (Kruger and Witkovsky, 1961; Goldby and Robinson, 1962; Ebbesson, 1967, 1969; Joseph and Whitlock, 1968b). The bulk of ascending spinal pathways to the brain stem and diencephalon, however, passes by way of the superficial zone of the lateral funiculus (Ebbesson, 1967, 1969; ten Donkelaar and Nieuwenhuys, 1979).

The dorsal root projections have been studied with the aid of normal (Chapter VI) and experimental (Chapter VII) material. Anterograde degeneration techniques (Nauta and Gygax, 1954; Fink and Heimer, 1967) have been used in all reptiles studied to trace the ensuing fiber degeneration following dorsal root transections. In the turtles *Pseudemys scripta elegans* and *Testudo hermanni*, also a recently developed technique has been used to study the distribution of dorsal root fibers within the spinal cord (Proshansky and Egger, 1977; Light and Perl, 1977): the enzyme horseradish peroxidase (HRP) has been applicated to the proximal stump of the transected dorsal root.

Our knowledge concerning the propriospinal pathway in reptiles is very limited. They are situated in the inner zone of the spinal white matter, whereas the paths connecting the spinal cord with higher levels of the central nervous system occupy the peripheral zone of the spinal cord. In mammals, a subdivision of the propriospinal pathways into short and long fiber contingents can be made, the latter interconnecting the cervical and lumbosacral intumescences. These long reciprocal propriospinal tracts are of great importance for the coordination of forelimb and hindlimb movements (cf. e.g. Miller and van den Burg, 1973). Part of the present thesis deals with the important question, whether in quadrupedal reptiles as lizards and turtles, which move their limbs in a particular diagonal pattern (cf. e.g. R.C. Snyder, 1952; Bellairs, 1970; Guibé, 1970; Zug, 1971; Walker, 1973; Sukhanov, 1974), such long reciprocal connections also exist. The neural elements necessary for interlimb coordination have been studied so far with physiological techniques in various reptiles, especially the turtle *Pseudemys scripta elegans* (Shimamura, 1973; Lennard and Stein, 1977; Stein, 1978) and with the HRP retrograde tracer technique in the lizard *Lacerta galloti* (ten Donkelaar and de Boer - van Huizen, 1978b).

In snakes a different condition is present. Snakes can move in several different ways, of which lateral undulation, concertina movement, rectilinear progression and sidewinding are probably the most common methods of locomotion (Gans, 1966; Bellairs, 1970; Guibé, 1970). The way in which the spinal cord controls these complex locomotor patterns is entirely unknown. However, it seems likely that short propriospinal fibers are more important for these movements than long propriospinal fibers. In the present study the propriospinal connections have been analysed with anterograde degeneration techniques as well as with the HRP tracer technique. The ascending spinal pathways to the brain stem and diencephalon have been studied with the anterograde degeneration techniques described above (Chapter IX). The descending supraspinal pathways have been shown to arise from the brain stem and from the hypothalamus (Robinson, 1969;

Cruce, 1975, 1979; ten Donkelaar, 1976a, b; ten Donkelaar and de Boer - van Huizen, 1978a). A projection from the telencephalon comparable to the mammalian corticospinal tract is not present in reptiles (cf. e.g. Lohman and van Woerden - Verkley, 1976; Hoogland, 1977). In the present study in particular some results with the HRP-technique in the reptiles studied will be shown (Chapter IX). By injecting the enzyme HRP the cells of origin of pathways descending to the spinal cord have been demonstrated.

Throughout the present study, differences in spinal organization between reptilian species with different locomotor patterns are emphasized and an attempt is made to compare the organization of the spinal cord of reptiles with that in other terrestrial vertebrates, particularly the amniotes.

For the description of the cytoarchitecture of the spinal gray, transverse series of the spinal cord of each reptile studied were used, stained with cresylechtviolet. The analysis of the fiber pattern is based on series stained according to Häggqvist's (1936) modification of the Alzheimer Mann methylblue-eosin stain. Further material for reference included series stained according to Klüver and Barrera (1953).

For the experimental part of this thesis altogether 32 lizards (22 *Tupinambis nigropunctatus* and 10 *Varanus exanthematicus*), varying in weight from 500 to 3000 grams, with a total length of 65 to 95 cm and a snout-vent length of 25 to 35 cm; 50 turtles (34 *Testudo hermanni*, 14 *Pseudemys scripta elegans* and 2 *Pelomedusa subrufa*), 150 to 1300 grams, with a carapace length ranging from 10 to 25 cm; and 21 snakes (*Python reticulatus*), 500 to 1500 grams, with a snout-vent length of 100 to 125 cm.

All experiments were carried out under surgical anaesthesia. The animals were intubated and received endotracheal anaesthesia for which a mixture of 0,5 l oxygen, 50 - 100 ml nitrous oxide with ¼ - ½ volumen-% halothane was employed. The operations were performed under sterile conditions (except for the turtle, where this is hardly necessary) with the aid of a Zeiss binocular operation microscope. The experiments carried out can be divided into 3 groups:

(1) *Dorsal rhizotomies*: In 12 lizards (*Tupinambis nigropunctatus*) and 4 snakes (*Python reticulatus*) following a midline skin incision and separation of the bilateral dorsal musculature a small laminectomy was performed for the adequate visualization of a single dorsal root ganglion. In 13 turtles (*Testudo hermanni*) a small opening was drilled in the carapace. Single dorsal roots were then sectioned either intradurally or extradurally, at a point immediately proximal to the ganglion. In the turtles *Testudo hermanni* (2 experiments) and *Pseudemys*

scripta elegans (6 experiments) in addition dry horseradish peroxidase was applicated to the cut end of the proximal stump of the transected dorsal root (modified after Proshansky and Egger, 1977).

(2) *Hemicordotomies*: Following laminectomy and incision of the dura a complete or partial spinal hemisection was performed with a von Graefe cataract knife in 11 lizards (8 *Tupinambis nigropunctatus* and 3 *Varanus exanthematicus*), 11 turtles (*Testudo hermanni*) and 12 snakes (*Python reticulatus*). These operations were carried out at various levels of the spinal cord. In the turtle *Testudo hermanni* also in two specimens successive hemisections were done.

(3) *Injections of the enzyme horseradish peroxidase (HRP) into the spinal cord*: Following laminectomy and incision of the dura, the enzyme horseradish peroxidase (HRP, Boehringer) was injected under control of the operation microscope into the spinal cord, viz., into the cervical and lumbar intumescences of lizards and turtles, and into various levels of the spinal cord of snakes. The enzyme HRP was dissolved in physiological saline in a concentration of 200 μg per μl (a 20% solution). In 9 lizards (2 *Tupinambis nigropunctatus* and 7 *Varanus exanthematicus*), 14 turtles (6 *Testudo hermanni* and 8 *Pseudemys scripta elegans*) and 5 snakes (*Python reticulatus*) 3 - 8 unilateral injections of 0,1 μl were made with a glass micropipette attached to a Hamilton syringe following a technique applied in mammals by Kuypers and Maisky (1975) and Molenaar and Kuypers (1975, 1978): the series of injections damaged many axons, and damaged fibers as well as terminals take up HRP and transport this enzyme retrogradely to their cell bodies. In 2 turtles (*Pelomedusa subrufa*) one injection of 0,2 μl was made into the gray matter of the lumbar intumescence.

Following surgery, the animals were kept at an environmental temperature ranging from 24° to 27°C (the snakes 27° to 30°C) and sacrificed after postoperative survival times of 10 to 40 days for reptiles in which a dorsal rhizotomy or a hemicordotomy was performed, and of 2 to 7 days for the reptiles in which HRP was injected into the cord or applicated to the transected dorsal root.

The reptiles used in the lesion experiments (i.e. the dorsal root transections and the spinal hemisections) were perfused transcardially under deep Nembutal anaesthesia, with physiological saline, followed by 10% formalin. After their removal, the brain and the spinal cord were further fixed in 10% formalin for periods varying from 2 to 10 weeks.

The material obtained from the dorsal root transections and the spinal hemisections was, in order to study the ensuing anterograde fiber and terminal degeneration, embedded in albumine, sectioned transversely on a freezing microtome at 25 µm thickness and subsequently stained with the Nauta-Gygax (1954) and Fink-Heimer (1967) techniques. The degeneration was charted either on outlines of sections drawn with the aid of a microprojector or directly using a Zeiss camera lucida attached to a microscope.

The brains and spinal cords of the animals in which HRP was injected into the spinal cord or applicated to the proximal stump of the transected dorsal root, were processed as follows. These reptiles were perfused transcardially after survival times of 2 to 7 days with a mixture of 1% formaldehyde and 1,25% glutaraldehyde in 0,1 M phosphate buffer (pH 7,4). The brain and the spinal cord were removed and stored overnight in cold phosphate buffer containing 30% sucrose. The material was frozen in dry ice and cut into sections of 40 µm in the transversal plane on a freezing microtome. The sections were incubated according to Graham and Karnovsky (1966) in a medium containing hydrogenperoxide and 3.'3-diamino benzidine tetrahydrochloride in tris-HCl buffer (pH 7,6) for 10 minutes at room temperature, and mounted in Entellan. Part of the sections was counterstained with cresylechtviolet. In addition to this classical HRP-visualizing technique (Kristensson and Olsson, 1971) also recent modifications (Hanker et al., 1977; Hardy and Heimer, 1977; Mesulam, 1978) have been used. These new HRP-staining techniques make use of different non-carcinogenic chromogens, viz. the Hanker-Yates reagent (Hanker et al., 1977) and tetramethylbenzidine (Hardy and Heimer, 1977; Mesulam, 1978). Particularly the latter substrate gives a reaction-product with superior sensitivity.

The reptiles studied show profound differences with regard to shape and development of the trunk, tail and extremities. These differences are clearly reflected in the gross structure of the spinal cord (Fig. 1). In forms without extremities, such as snakes and limbless lizards, the cord lacks cervical and lumbar enlargements, but these swellings are well marked in turtles, lizards and crocodiles, i.e. cervical and lumbar intumescences occur in the spinal cord related to the development of limbs. This relation is further indicated by the presence of a large lumbar enlargement in reptiles as e.g. the lizard *Tupinambis nigropunctatus*, which possess strongly developed posterior extremities. In this respect it is interesting to note that in some large fossil dinosaurs, which possessed large posterior extremities, the cavity within the vertebral canal occupied by the lumbar intumescence exceeds the volume of the endocranial cavity.

An ascencus medullae does not occur in reptiles, i.e. their spinal cord extends throughout the entire length of the vertebral canal, nearly reaching the end of the tail (Ariëns Kappers et al., 1936; Nieuwenhuys, 1964; Kuhlenbeck, 1975). The relative length of the spinal cord may correlate with the retention in the reptilian tail, of a 'primitive' muscular metamerism, which becomes superseded or fades out in mammals (Kuhlenbeck, 1975).

An established nomenclature for the different regions of the reptilian medulla spinalis does not exist. In part this absence of a clear terminology is due to the fact that reptilian vertebrae are morphologically more uniform than their mammalian counterparts. A few remarks about the reptilian vertebral column, based largely on a recent and very extensive discussion of this structure by Hoffstetter and Gasc (1969) may be appropriate here.

In the order *Testudines* there are generally 18 presacral

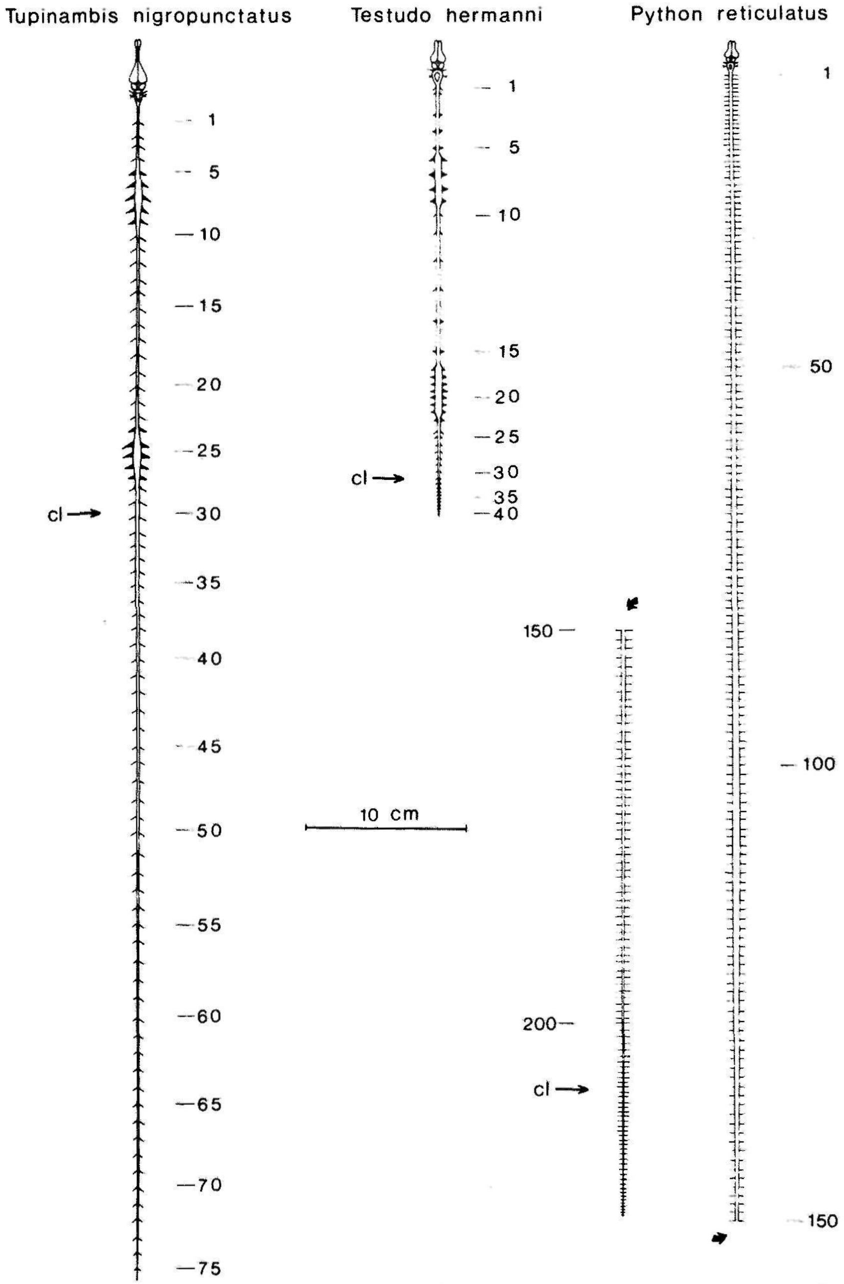


Fig. 1 Schematic representation of the central nervous system in *Tupinambis nigropunctatus*, *Testudo hermanni* and *Python reticulatus*, showing, in particular, the number and distribution of spinal segments. Abbreviation: cl, level of cloaca.

vertebrae. Despite the lack of a sternum, a cervical region, consisting of eight movable elements, and a trunk region are easily distinguished within the vertebral column. Exceptions to the rule, i.e. cases of nine cervicals, are due to individual variations. In the trunk the 10 dorsal vertebral bodies are ankylosed, and the well developed ribs are connected to the dermal carapace. In addition to these 18 presacral vertebrae, two sacral vertebrae are present in *Testudines*. Their tail comprises about 16 elements.

In lizards, the number of presacral (or precloacal) vertebrae varies widely. Some families, species of which are frequently used in present-day experimental neuroanatomical research, will be discussed here; namely the *Iguanidae* (e.g. *Iguana iguana*), the *Teiidae* (e.g. *Tupinambis nigropunctatus*) and the *Varanidae* (e.g. *Varanus exanthematicus*). In iguanids, the number of presacral vertebrae varies from 22 - 28 (with 24 as modal value), whereas in teids (tetrapod species), a total of 24 - 27 (with 26 as modal value) precloacal vertebrae is present. Varanids possess presacral vertebrae varying from 28 - 30 (with 29 as modal value). Since the time of Cuvier and especially after the work of Stannius (1849), cervical vertebrae have been defined as those preceding that bearing the first rib united to the sternum. All other definitions offer too many exceptions or contradictions to be adopted (Hoffstetter and Gasc, 1969). There are typically eight cervical vertebrae in iguanids and teids. Different numbers have been registered as individual variations, however, varanids possess nine cervical vertebrae with a concomitant backward displacement of the brachial plexus. In teids and varanids, there is only one true lumbar vertebra, i.e. a caudal presacral vertebra which lacks completely ribs. Two sacral vertebrae supporting the pelvis are present. In *Tupinambis nigropunctatus*, some 50 caudal (or tail) elements can be distinguished.

In snakes, the precloacal region is quite uniform. The absence of a pectoral girdle in all snakes makes it impossible to refer to the site of this organ in defining the boundary between the cervical and trunk regions. The vertebral number in snakes is always high, ranging from 160 - 400. Of these, 120 to over 320 are precloacal. In the species *Python reticulatus* studied in the present investigation, some 200 - 220 precloacal elements were present, whereas in the short tail about 30 caudal (or tail) vertebrae could be distinguished.

Surveying these variations in the reptilian vertebral column, i.e. in number as well as in composition of vertebrae, a nomenclature for the segments of the spinal cord, as is customary in mammals, seems inappropriate at the time. Therefore, and to avoid any confusion, the spinal segments will be numbered sequentially from the first cervical one.

In *Tupinambis nigropunctatus*, the roots of the 6th - 9th segments enter the brachial plexus, whereas the roots of the 24th - 28th segments enter the lumbosacral plexus (Cruce, 1979). The brachial plexus of the turtle *Testudo hermanni*, is made up of the spinal roots arising from the 7th - 10th segments; the spinal roots of the 19th - 22nd segments enter the lumbosacral plexus.

IV GENERAL RELATIONS OF THE GRAY AND WHITE MATTER, AND CHARACTERISTICS OF THE VARIOUS REGIONS OF THE SPINAL CORD

Several longitudinal furrows are seen on the surface of the cord (Fig. 2). A deep anterior median fissure and a shallow posterior median sulcus are easily recognized on the spinal cords of all the reptiles studied. The presence of anterolateral and posterolateral grooves is not obvious. Especially in the lizard *Tupinambis nigropunctatus* and in the snake *Python reticulatus*, a shallow groove is present on the lateral surface of the cord in the area where the so-called marginal nuclei (Fig. 2 and Chapter V) are situated. Moreover, it should be noted that the spinal cord at this region is closely attached to its meninges.

A cross section of the spinal cord shows a central four-horned area of gray matter surrounded by a much larger area of white matter. The boundary between gray and white matter, although more distinct than in fish, is less definite than in birds and mammals (Nieuwenhuys, 1964). The gray matter of the reptilian cord shows a clear division into ventral and dorsal horns. The dorsal horns separate off a portion of the white matter, the so-called dorsal funiculus. The remainder of the white matter can be subdivided into lateral and ventral funiculi, using the intraspinal trajectory of the ventral root fibers as a landmark. Since an intermediate or lateral horn is absent in the reptilian spinal gray, a subdivision of the lateral funiculus into posterolateral and anterolateral regions, as is customary in mammals, cannot be made in this class.

Figure 2 shows diagrams of transverse sections of representative spinal cord segments, namely through the cervical and lumbosacral enlargements completed with a mid-thoracic section, a high cervical section and tail segments in the lizard *Tupinambis nigropunctatus*, and the turtle *Testudo hermanni*, whereas in the snake *Python reticulatus*, the 12th, the 56th, the 110th (about in the middle of this animal), the 170th and

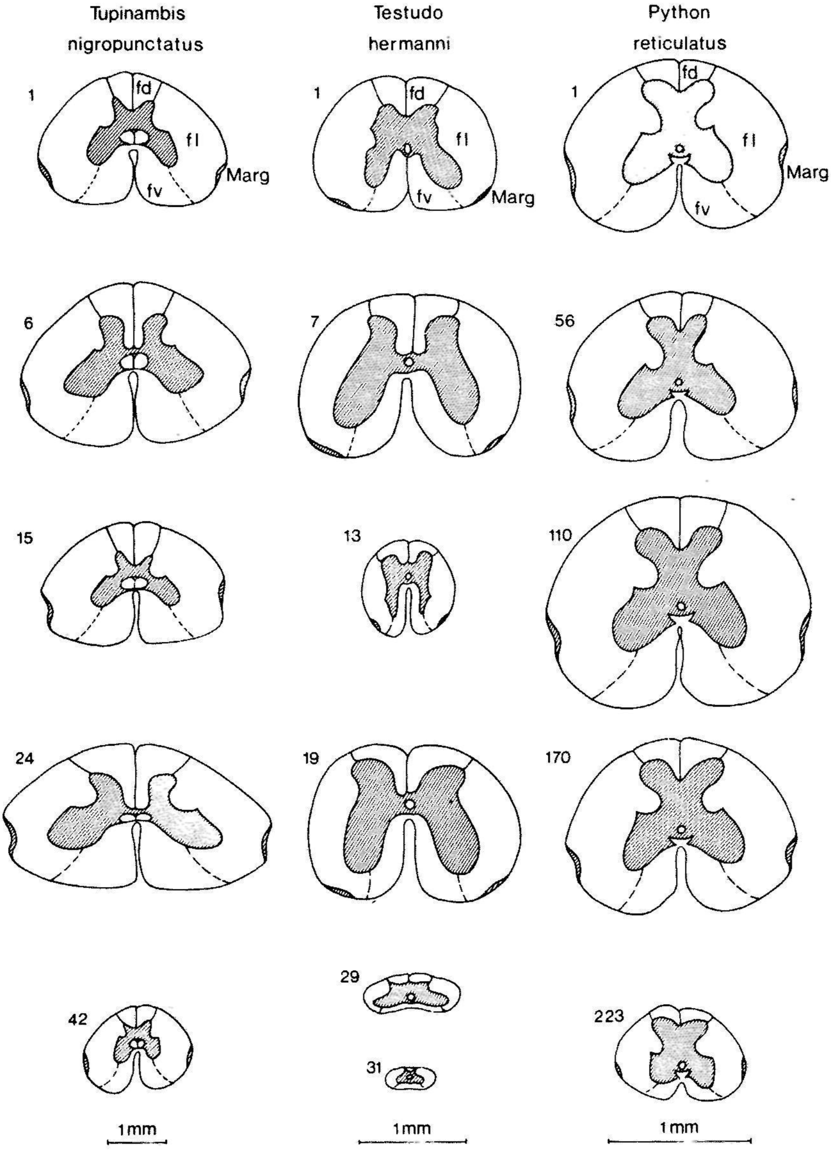


Fig. 2 Diagrammatic representation of transverse sections through representative levels of the spinal cord of *Tupinambis nigropunctatus*, *Testudo hermanni* and *Python reticulatus*, showing the variations in the size of the cord. Abbreviations: fd, funiculus dorsalis; fl, funiculus lateralis; fv, funiculus ventralis; Marg, nucleus marginalis.

the 223rd spinal segments have been selected. The size and shape of the spinal cord and the relative proportion of the gray and white matter vary greatly. Several factors appear to be responsible for these differences (Ranson and Clark, 1959). The variation in size of the nerve roots at the various levels of the spinal cord may be significant. At levels where great numbers of nerve fibers enter the spinal cord, particularly in the intumescences, they cause a clear increase in the size of that structure. All levels of the spinal cord are connected with the brain stem by bundles of long ascending as well as descending fibers. The long ascending fibers increase in number rostrally and, therefore, might cause an increase in the cross-sectional area of the white matter. This phenomenon is known as <u>frontal accumulation</u>; it is reported to be 'clearly evident' in reptiles (Ariëns Kappers et al., 1936). A third factor responsible for differences in size of the spinal cord, namely the influence of propriospinal fibers, will be discussed later.

The descending pathways might conceivably behave in a comparable way by showing a gradual decrease in the rostrocaudal direction. However, it should be noted that recent physiological experiments have revealed that, in the cat axons of various descending pathways, namely the vestibulospinal tract (Abzug et al., 1973, 1974), the reticulospinal tract (Peterson et al., 1975), the rubrospinal tract (Shinoda et al., 1977), and the corticospinal tract (Shinoda et al., 1976) do show a considerable <u>branching</u> during their spinal course. In reptiles there is no direct evidence for collateral branching of descending fibers. However, the observation (ten Donkelaar, 1976a) that retrograde cell changes in the reticular formation of the brain stem can be observed following cervical lesions, whereas such cell changes are lacking following lumbar lesions, renders it likely that collateral branching of descending pathways is also present in reptiles.

In order to obtain more precise information on frontal accumulation in reptiles the following technique, previously used in the ostrich *Struthio camelus* (Streeter, 1904) and in various mammals (e.g. the opossum *Didelphis virginiana*, Voris, 1928; man, Donaldson and Davis, 1903), has been employed. Two sections were selected from the middle of each segment in the three reptiles studied. Outline drawings of the sections were prepared with a Bausch and Lomb microprojector and the gray matter, the dorsal funiculus as well as the ventral and lateral funiculi, have been indicated. The cross-sectional areas of the entire section, the gray matter, the dorsal funiculus as well as the ventral and lateral funiculi were determined planimetrically. The areas of the ventral and lateral funiculi were combined. Data from two sections have been averaged and plotted for the lizard *Tupinambis nigropunctatus* (Fig. 3A), the snake *Python reticulatus* (Fig. 3B) and the turtle *Testudo hermanni* (Fig. 4).

Discussion and conclusions:

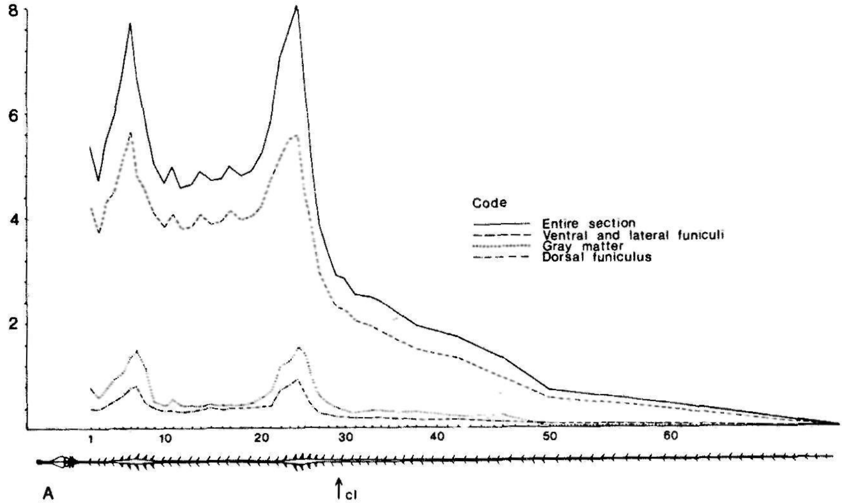
The diagrams (Figs. 3, 4) based on the planimetrical data indicated in the previous section allow the following conclusions:

1) The variations in size of the spinal cord are particularly due to changes in the ventral and lateral funiculi. These funiculi form by far the greatest area at all levels of the spinal cord.

2) The curves which represent the cross-sectional areas of the gray matter and the dorsal funiculi show a close uniformity in size, although the former shows a greater increase in the enlargements.

3) In *Tupinambis nigropunctatus* and *Testudo hermanni*, the area of the gray matter is remarkably constant in the 'thoracic' part of the spinal cord, i.e. between the intumescences.

4) In both the cervical and lumbosacral enlargements of the lizard *Tupinambis nigropunctatus* and the turtle *Testudo hermanni* the increase in area of the ventral and lateral funiculi is greater than that of the gray matter and dorsal funiculus. This is probably due to the large number of propriospinal



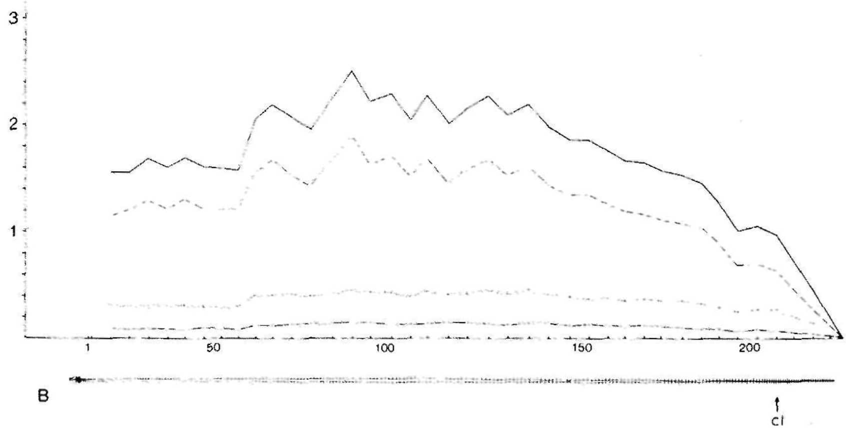


Fig. 3 Graphs of cross-sectional areas in *Tupinambis nigropunctatus* (A) and *Python reticulatus* (B). The spinal segments are marked to scale on the abscissa, whereas the cross-sectional areas are represented on the ordinates in square millimeters. Abbreviation: cl, level of cloaca.

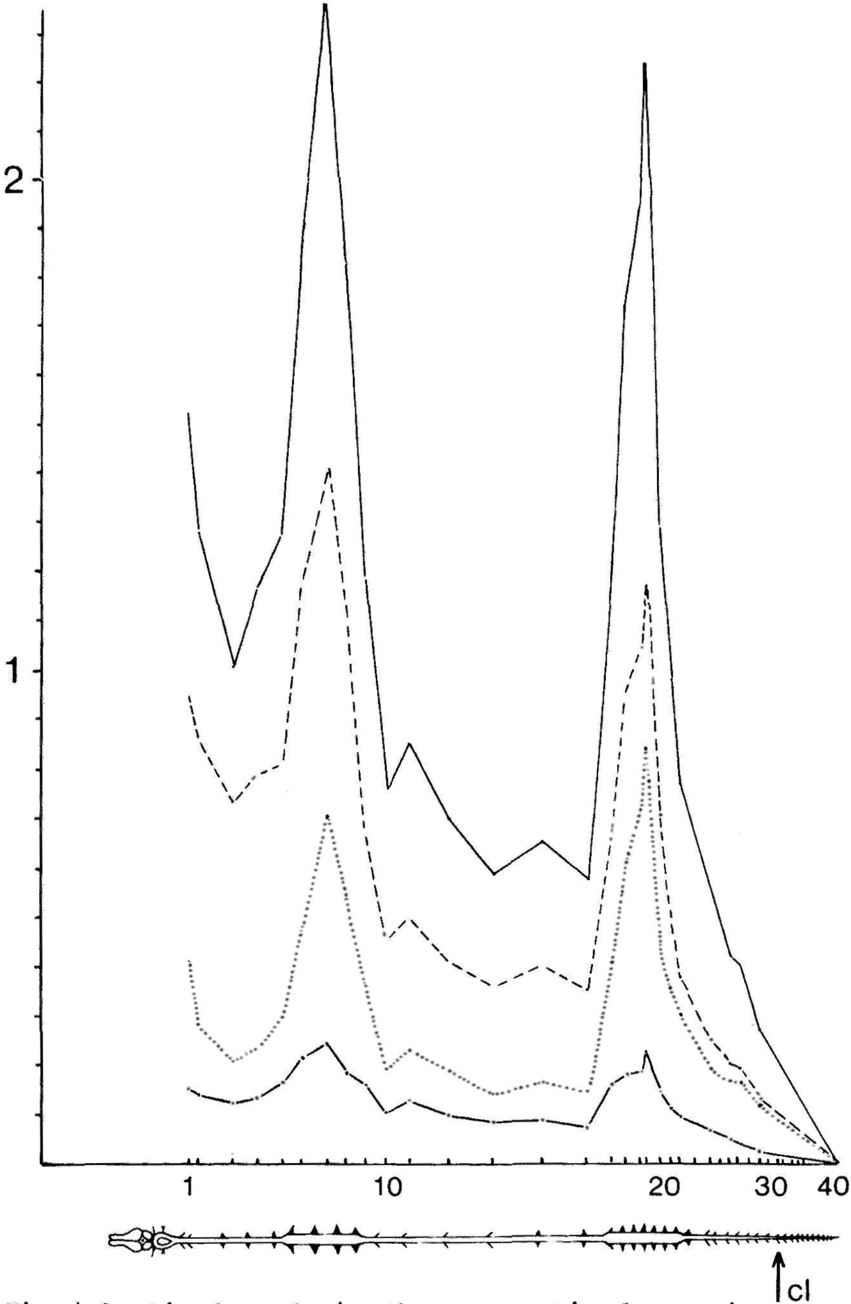


Fig. 4 Graphic chart showing the cross-sectional areas in *Testudo hermanni*. For code see figure 3. Abbreviation: cl, level of cloaca.

fibers in the intumescences.

5) The dorsal funiculi are the least variable. The dorsal funiculus curve shows an abrupt rise near the entrance of the large dorsal roots related to the extremities. These data suggest that the course of the dorsal root fibers is a short one and that only a small proportion of these fibers reach the brain stem by way of the dorsal funiculus.

6) The presence of frontal accumulation in the dorsal funiculus is evident in *Testudo hermanni* (Fig. 4). However, this phenomenon could not be demonstrated in the other reptiles studied. Goldby and Robinson (1962) also remained unable to prove the existence of frontal accumulation in the lizard *Lacerta viridis*. The latter authors supposed that variations in the number of the abundant propriospinal fibers present at different levels, mask the effects of additions from each segment of a few long ascending fibers.

7) An even more pronounced frontal accumulation is present in the remainder of the white matter in *Testudo hermanni*. It seems likely that this phenomenon is due to long ascending and descending fibers.

8) In *Python reticulatus* (Fig. 3B) an enlargement of the spinal cord is present, which is clearly related to the well-developed main part of its trunk. This intumescence may be designated as the <u>intumescentia trunci</u>. It is most pronounced in the curve of the ventral and lateral funiculi, but the gray matter also shows a clear increase in cross-sectional area. The curves are flat in the 'neck'-area. Therefore, the trunk enlargement of *Python reticulatus* is not due to an increase in pathways connecting the spinal cord with the brain stem, but to a profuse development of propriospinal fibers.

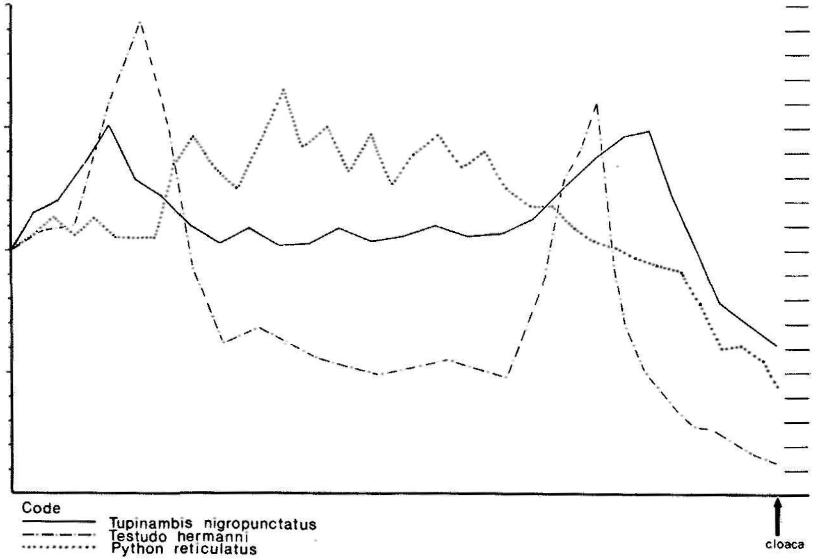


Fig. 5 Diagram showing the cross-sectional areas of the ventral and lateral funiculi in *Tupinambis nigropunctatus*, *Testudo hermanni* and *Python reticulatus*. The intumescence associated with the trunk in *Python reticulatus* is situated between the enlargements related to the extremities in the other reptiles studied.

Finally, in figure 5 a comparison has been made of data concerning the cross-sectional areas of the ventral and lateral funiculi. The variations in area in these funiculi are representative for the entire spinal cord. This diagram shows clearly that the intumescence associated with the trunk in *Python reticulatus* is situated between the enlargements related to the extremities in the other reptiles studied.

The gray matter of the reptilian cord shows a clear division into dorsal and ventral horns. The dorsal horn can be characterized as having a dorsal root input, whereas the ventral horn contains the motoneurons. A large intermediate zone remains between dorsal and ventral horns, which consists mainly of interneurons.

Subdivisions of the reptilian spinal gray have been provisional so far (Ariëns Kappers et al., 1936; Nieuwenhuys, 1964; ten Donkelaar, 1976b). For experimental neuroanatomical as well as for physiological studies of the reptilian spinal cord a more detailed analysis of the gray matter is necessary. The most widely adopted scheme for subdivision of the spinal gray stems from extensive cytoarchitectonic studies in cat (Rexed, 1952, 1954, 1964). Figure 6 shows the classical subdivision of the gray matter as well as Rexed's parcellation of the spinal gray into ten laminae. The same principles of laminar organization are considered to apply to the spinal cord of 'lower' (e.g. opossum: Martin and Fischer, 1968; rat: McClung and Castro, 1978) as well as 'higher' mammals (Rexed, 1964). Recently, Rexed's subdivision has also been applied to the cord of non-mammalian vertebrates, namely the pigeon *Columba livia* (Leonard and Cohen, 1975a) and the lizard *Tupinambis nigropunctatus* (Cruce, 1975, 1979).

In the present thesis a subdivision is presented which clearly reflects the influence of Rexed's laminar approach, especially as regards the dorsal horn. The neutral term 'area' has been used, since not all cell groups are distinguishable as laminae. To facilitate comparison with Rexed's parcellation and to avoid confusion by introducing a different subdivision of the reptilian spinal gray, Rexed's approach and numbering convention will be followed as close as possible. The delineation of cell groups has been performed with the help of the usual cytoarchitectonic criteria (cf. e.g. Cruce and Nieuwenhuys,

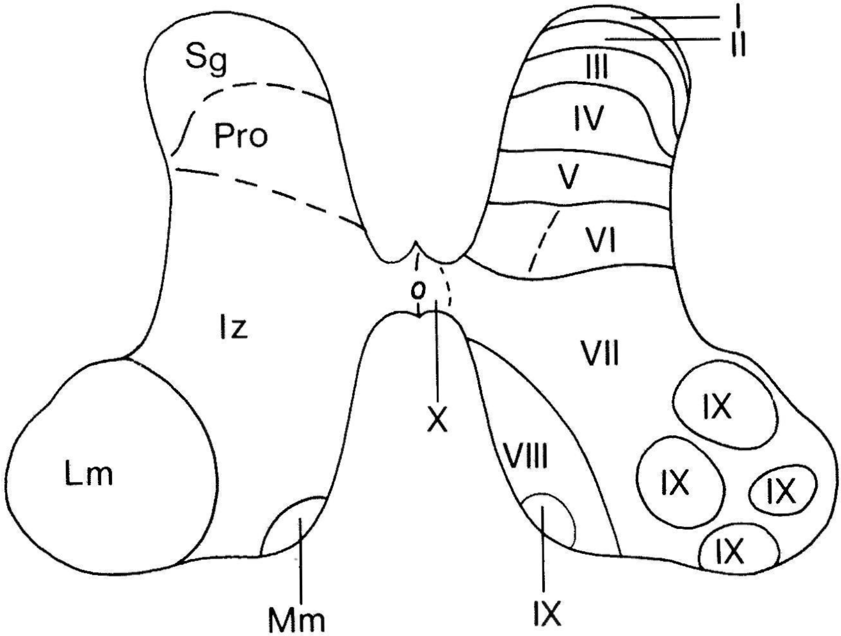


Fig. 6 Diagram showing the classical subdivision of the gray matter at the left as well as Rexed's parcellation of the cat spinal gray into ten laminae at the right (redrawn from Rexed, 1954). Abbreviations: Iz, intermediate zone; Lm, lateral column of motoneurons; Mm, medial column of motoneurons; Pro, nucleus proprius; Sg, substantia gelatinosa.

1974), i.e. (1) the size and shape of the somata, (2) the disposition of the Nissl substance, and (3) the density and the pattern of the cell arrangement. The cells in the spinal cord of the three reptiles studied show considerable differences in size, ranging from 4 to 60 µm. For convenience of description we have subdivided these cells into three categories: large (15 - 60 µm), medium-sized (10 - 15 µm) and small (4 - 10 µm).

The following description of the reptilian spinal cord is based primarily on Nissl-stained serial sections (Fig. 7). Additional material for reference included series stained according to Klüver and Barrera (1953). The various areas of the spinal gray will now be described for the lizard *Tupinambis*

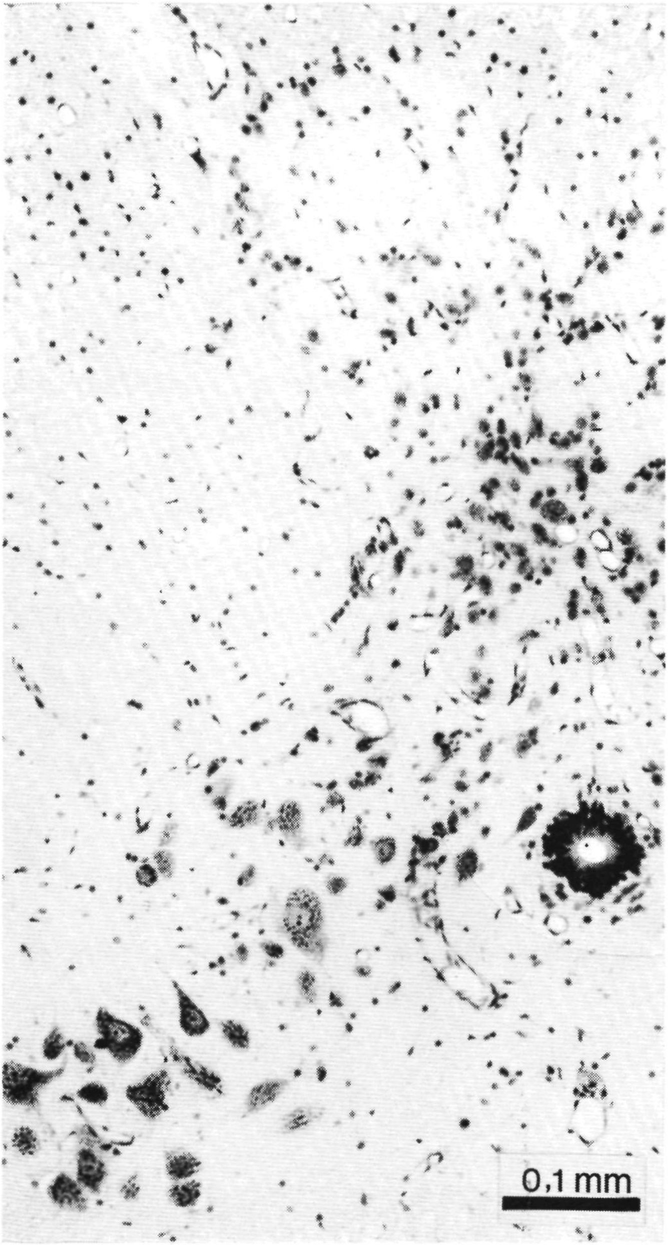


Fig. 7 Photomicrograph of a Nissl-stained transverse section showing the arrangement of the spinal gray in the 56th segment of the snake *Python reticulatus*.

nigropunctatus. Unless indicated otherwise, the description also applies to the turtle *Testudo hermanni*, and the snake *Python reticulatus*. In order to elucidate the description, diagrams of the gray matter of representative levels of the spinal cord of the three reptiles studied are presented in figures 8 and 9.

In the dorsal horn of *Tupinambis nigropunctatus* (Fig. 8) and *Python reticulatus* (Figs. 7, 9B), four more or less distinct cellular areas can be recognized, the first three of which show a distinct laminar arrangement. In *Testudo hermanni*, the subdivision of the dorsal horn is less clear than in the other reptiles studied (Fig. 9A).

Area I consists of a single layer of cells which caps the dorsal horn. This area extends ventrally along the medial and lateral aspects of the dorsal horn without reaching the base, however. Most of the neurons of this area are small and round or fusiform in shape. They have relatively large, clear nuclei with indistinct nucleoli. Their cytoplasm is restricted to a narrow perinuclear region with the Nissl substance concentrated close to the nuclear membrane. This area cannot be delineated in *Testudo hermanni*.

Area II is situated directly ventral to area I and follows its contours. It consists of small cells, which are rather loosely arranged. Most are spindle-shaped with round, clear nuclei. Their Nissl substance is sparse and surrounds the large clear nucleus. In the turtle *Testudo hermanni*, areas I and II could not be distinguished as separate entities, and therefore have been taken together. Area I - II contains medium-sized cells in this turtle, rather than the small cells present in areas I and II of the other reptiles studied.

In all reptiles studied, an almost cell-free zone is situated ventral to area II. This zone has been designated as *area III*.

Area IV constitutes the main part of the dorsal horn. Its ventral boundary is quite distinct since the cells of area V - VI show a characteristic arrangement which will be described

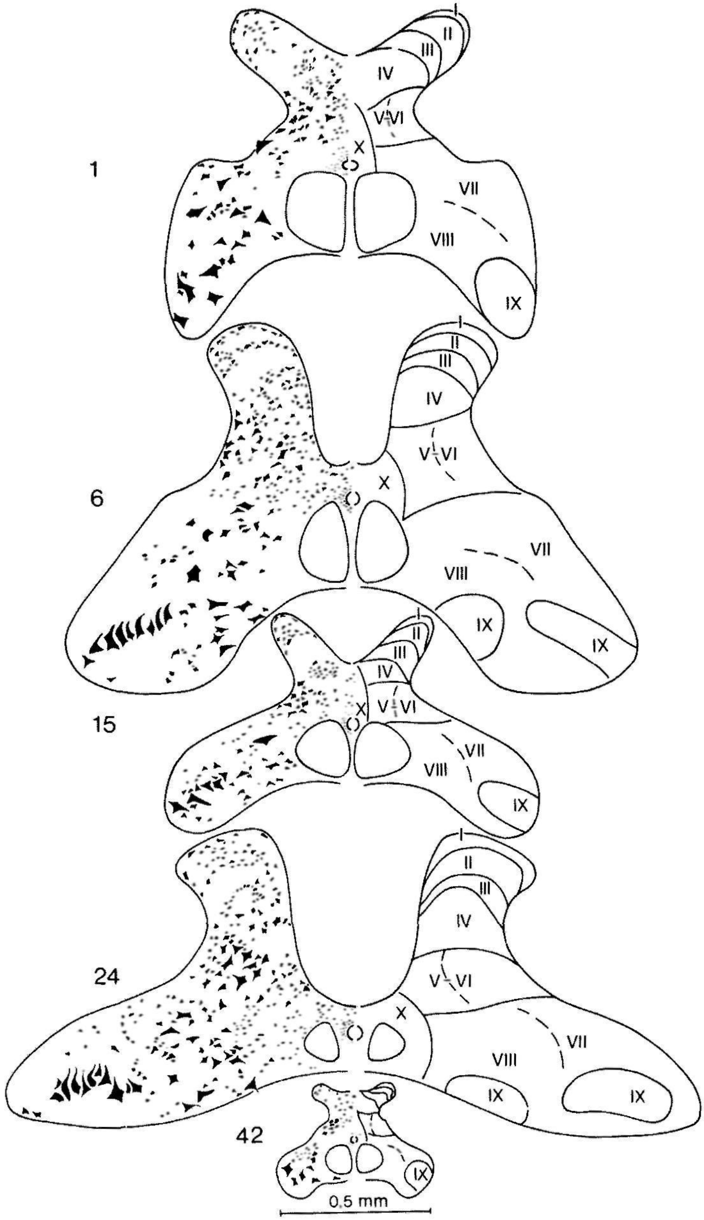


Fig. 8 Camera lucida drawings representing transverse sections through the spinal gray of *Tupinambis nigropunctatus*. At the left the cell picture, at the right the cytoarchitectonic regions outlined.

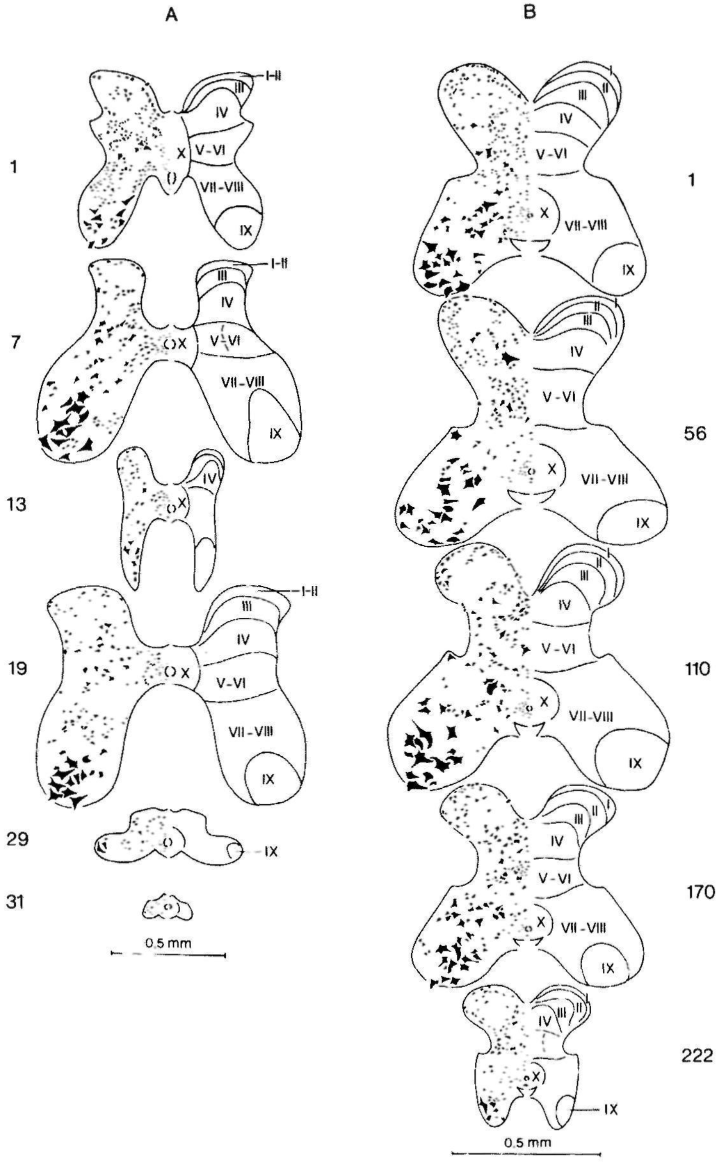


Fig. 9 Camera lucida drawings representing transverse sections through the spinal gray of *Testudo hermanni* (A) and *Python reticulatus* (B). At the left the cell picture, at the right the cytoarchitectonic regions outlined.

below. Area IV is a zone of very tightly packed, spindle-shaped, medium-sized cells. The neurons have round clear nuclei with dark nucleoli. In most rostral (2nd upwards) and caudal (28th downwards) segments area IV extends across the midline dorsal to area X. In *Python reticulatus*, area IV extends across the midline at all levels of the spinal cord and constitutes the wide dorsal gray commissure, characteristic for the cord of this animal. In *Testudo hermanni*, the border between area IV and area V - VI is rather indistinct. There is a gradual shift in cell size and packing density in these areas.

The remaining part of the spinal gray, i.e. the so-called base of the dorsal horn, the intermediate gray zone and the motoneurons in the ventral horn, do not show a clear laminar arrangement. The motoneuron groups (area IX) and a central zone (area X) form distinct areas. The large, remaining region can be subdivided into dorsal and ventral zones. The dorsal part consists of the base of the dorsal horn and the adjacent part of the intermediate zone. The ventral zone contains the main part of the intermediate zone. Rexed has subdivided this large region into four laminae (V to VIII). Comparison with mammals renders it likely that the dorsal part of the reptilian spinal gray mentioned above corresponds to Rexed's laminae V and VI, whereas the so-called ventral zone resembles Rexed's laminae VII and VIII. Therefore these two regions have been designated as area V - VI and area VII - VIII in the present study. In the thoracic region of *Testudo hermanni*, motoneurons are absent due to the lack of trunk musculature. Therefore, the ventral horns are extremely narrow. The cells occurring in the ventral horn of the turtle mentioned represent commissural cells, various interneurons and, possibly, cells of origin for preganglionic fibers (Ariëns Kappers et al., 1936).

Area V - VI forms a relatively wide zone which can be clearly distinguished from area IV due to distinct differences in cytoarchitecture and fiber pattern. This area is bounded on the medial side by the central area X, and ventrally by area VII - VIII. Area V - VI can be divided into lateral and medial zones. The lateral zone contains large polygonal cells (15 - 30 um) with round to spindle-shaped nuclei and clear, deeply stained nucleoli. They possess abundant Nissl substance scattered throughout the cytoplasm. The lateral zone is traversed by bundles of entering dorsal root fibers which create a reticulated appearance. The medial zone contains scattered, small lightly staining cells (6 - 10 um). Their Nissl substance has a rough granular aspect, spreading throughout the cytoplasm without any characteristic arrangement. In *Python reticulatus*, subdivision of area V - VI into lateral and medial zones can also be made at most levels. In *Testudo hermanni*, however, these two zones can only be recognized in the cervical intumescence.

Area VII - VIII contains numerous large polygonal cells (20 - 30 um) with round and distinct nucleoli. These large neurons are particularly situated medially in the ventral horn. The Nissl substance is of a fine, granular appearance and equally scattered throughout the cytoplasm. Between these typical cells for this area also round to oval small cells with round nuclei and distinct nucleoli are present. Their Nissl substance is situated at the periphery of the cytoplasm. In *Tupinambis nigropunctatus*, at least in the intumescences, the area in question can be divided into a dorsolateral (area VII) and a ventromedial (area VIII) zone. This distinction is based on the heterogeneous cell population in the ventromedial part of area VII - VIII, which ranges from small cells to cells as large as motoneurons (Cruce, 1975, 1979). The lateral border of area VIII with area VII is indistinct. In *Python reticulatus* and *Testudo hermanni*, a subdivision of area VII - VIII as in the tegu lizard, cannot be made.

Area IX, i.e. the motoneuron area, consists in the lizard *Tupinambis nigropunctatus* of two longitudinal columns, a medial and a lateral one. The medial column, which is present throughout the spinal cord, is related to the innervation of neck, trunk and tail musculature. The lateral column is present only in the cervical and lumbosacral enlargements and is related to the innervation of the extremity muscles (Ariëns Kappers et al., 1936; Nieuwenhuys, 1964). Motoneurons are large (30 - 60 um), darkly staining cells, which are multipolar in shape with large, round nuclei and clear, deeply staining nucleoli. They possess abundant Nissl substance scattered throughout their cytoplasm. In *Tupinambis nigropunctatus* the cells of the lateral column show a palisade-like arrangement. In *Testudo hermanni* motoneurons are present throughout the spinal cord except for the thoracic region. Two major subdivisions are found, a more medial group consisting of motoneurons innervating neck and tail musculature, and a more lateral group, which is present only in the enlargements. In *Python reticulatus* the motoneurons constitute a single continuous column, which is particularly large in the intumescentia trunci. These motor cells are probably comparable to the medial column described above for the other reptiles studied. Some large neurons located within area IX may not be motoneurons, since in a HRP-study in the lizard *Lacerta galloti* (ten Donkelaar and de Boer - van Huizen, 1978b) large neurons in area IX were found which project to the brain stem.

Area X, which surrounds the central canal, can be distinguished throughout the spinal cord. This area, also called substantia grisea centralis, is composed of very small (4 - 6 um) round cells with round nuclei and indistinct nucleoli. These cells have a very narrow rim of cytoplasm which does not contain discrete Nissl substance.

Within the spinal gray of the reptilian spinal cord various types of cells are found, which are designated (cf. e.g. Banchi, 1903) as funicular cells, commissural neurons and motoneurons. Among the funicular and commissural neurons there are large, elongated elements which send numerous dendrites into the white matter, but the dendritic trees of others are much more restricted and confined to the gray substance (Banchi, 1903). Many of the secondary neurons in the reptilian spinal cord send one of their dendrites across the midline via a commissure situated dorsal to the central canal. This commissure, which will be called the dorsal gray commissure, contains a number of spindle-shaped neurons, the so-called dorsal median cells (Cajal, 1891; Banchi, 1903). Cruce (1979) described cells with dendrites confined to his laminae V to VII (cf. Fig. 10) which send their axons into the ipsilateral white matter or through a commissure ventral to the central canal to the contralateral side. These elements possibly represent the cells of origin of long ascending pathways. The cells of origin of various long ascending pathways have been localized in mammals with the horseradish peroxidase technique (Chapter IX). The shape of such neurons can be studied with the newly developed intracellular staining techniques (Kater and Nicholson, 1973; Jankowska, 1975).

The reptilian cord contains, besides these elements with long axons, true internuncial cells of Golgi's type II in the dorsal horn (Banchi, 1903). They are provided with a rather restricted, but richly ramifying dendritic tree and their axons branch ventral to the cell body in the basal part of the dorsal horn.

Whether in the reptilian spinal gray a column of Clarke, giving rise to the dorsal spinocerebellar tract as in mammals, is present, could not be convincingly demonstrated. In birds (van den Akker, 1970; Leonard and Cohen, 1975a) a column of large cells is present in layer V (Leonard and Cohen, 1975a) which extends beyond thoracic and upper lumbar segments. Moreover, van den Akker (1970) considers the dorsal position of the above-mentioned column as further cause for reservation since Rexed's (1952) assertion that Clarke's column always lies in lamina VII. For several reasons, however, Leonard and Cohen (1975a, b) consider the dorsal magnocellular column in lamina V in the pigeon as the avian homologue of Clarke's column in mammals. The large-celled lateral part of the reptilian area V - VI may be a candidate for a reptilian equivalent of the longitudinal structure mentioned above in the pigeon. However, in a recent experimental study using the horseradish peroxidase

(HRP) technique (ten Donkelaar and de Boer - van Huizen, 1978b) no evidence for the existence of a column of Clarke has been found in the lizard *Lacerta galloti*.

Preganglionic cells are undoubtedly present, but they are not arranged as a lateral horn like in mammals. It seems reasonable to assume that in the thoracic part of the spinal cord of the turtle these cells must occupy a fairly central position, since only the central portions of the gray substance are preserved in the thoracic region.

The motoneurons possess an extensive dendritic plexus. The medial group of motoneurons tends to elaborate its dendrites within area VIII (Cruce, 1979). The dendrites of the lateral column of motoneurons extend from the gray matter into the ventral and lateral funiculi. Two prominent dendrites are present (Banchi, 1903; Cruce, 1979), one extending medially and ventrally, the other extending dorsally along the medial border of the lateral funiculus. The latter sends numerous branches radially into the lateral funiculus. The terminal ramifications of these radial dendrites constitute a dense subpial plexus. Retrograde transport of the enzyme HRP via the transected ventral roots in the turtles *Pseudemys scripta elegans* and *Testudo hermanni* (cf. Chapter VII), resulted in the presence of labeled motoneurons in the ventral horn which possessed an extensive dendritic plexus (Fig. 21A). In the thoracic segments however, no labeled cells could be observed in the ventral horn.

No gamma-motoneurons are present in the reptilian spinal cord. The motor innervation of muscle spindles is derived from collaterals of axons which innervate extrafusal muscle fibers (Crowe and Ragab, 1970; Cliff and Ridge, 1973; Proske and Ridge, 1974; Ichiki et al., 1976).

A peculiarity of the reptilian spinal cord are accumulations of nerve cells, situated just beneath the pial surface dorsal to the ventral roots. These clusters, i.e. the nuclei marginales (Fig. 2), were first described by Gaskell (1885) in the alligator, and have been demonstrated in a wide variety of reptiles

(Ariëns Kappers et al., 1936). These outlying neurons, also called nuclei of Gaskell or nuclei of Hofmann - von Kölliker (von Kölliker, 1902), constitute a column extending throughout the spinal cord (Fig. 2). In the lizard *Tupinambis nigropunctatus* and the snake *Python reticulatus*, the marginal cells constitute easily recognizable clusters. In the turtle *Testudo hermanni*, however, only a few cells are scattered at the periphery of the cord. In all reptiles studied, the marginal cells are ovoid to multipolar in shape with relatively large nuclei, clear nucleoli and distinct Nissl substance. Besides these marginal nuclei, a number of cells varying in shape and size is scattered over the lateral funiculus.

Discussion

The present findings suggest that the spinal gray of the reptiles *Tupinambis nigropunctatus*, *Testudo hermanni* and *Python reticulatus* can be divided into a number of areas on a cytoarchitectonic basis. It should be stressed, however, that while some layers are clear, others are less so, and their delineation is somewhat arbitrary; a fact which has also been emphasized by other workers (e.g. Rexed, 1952; Leonard and Cohen, 1975a). In general, it may be stated that most boundaries between the areas in the dorsal horn are distinct, whereas the cellular elements of the more ventral areas are more diffusely arranged. In figure 10 diagrams showing the subdivision of the spinal gray in the three reptiles studied are presented. A closely corresponding parcellation of the spinal gray can be made for all species studied. In *Testudo hermanni* the differentiation of the spinal gray is less marked than in the other reptiles studied. In *Tupinambis nigropunctatus* Cruce (1975, 1979) has also described a laminar organization of the spinal gray (Fig. 10). The present data do differ substantially from those described by Cruce, particularly as regards the subdivision of the dorsal horn. The parcellation of the reptilian spinal gray presented in this thesis corresponds more closely to subdivisions made in other amniotes.

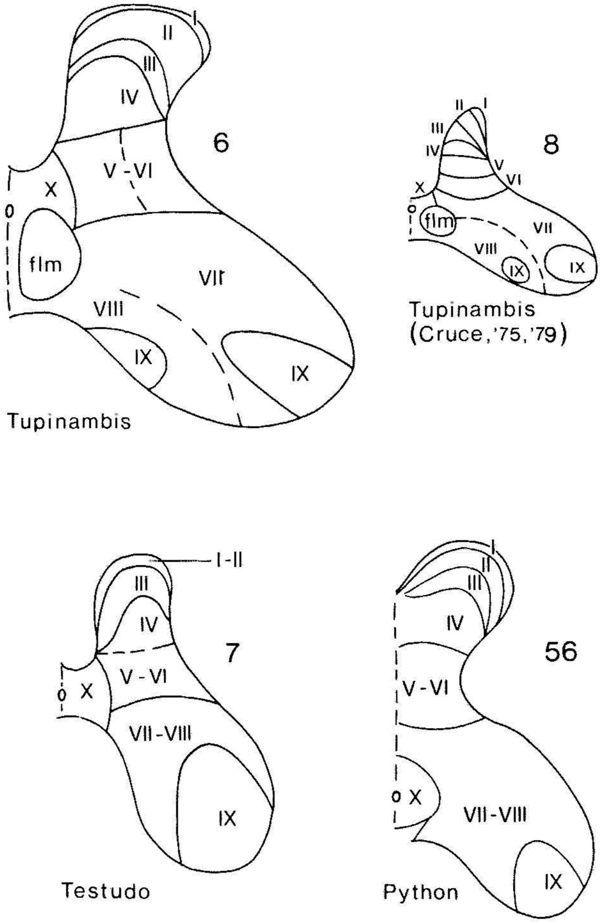


Fig. 10 Diagrams summarizing the subdivision of the reptilian spinal gray made in the present study. A diagram of the subdivision of the gray matter in *Tupinambis* (Cruce, 1975, 1979) has also been indicated. Abbreviation: flm, fasciculus longitudinalis medialis.

Finally, some comparative notes will be made on the laminar organization of the spinal gray in terrestrial vertebrates. In figure 11 diagrams are presented of the parcellation of the spinal gray in the frog *Rana catesbeiana* (Ebbesson, 1976a), the lizard *Tupinambis nigropunctatus* (the present study), the pigeon

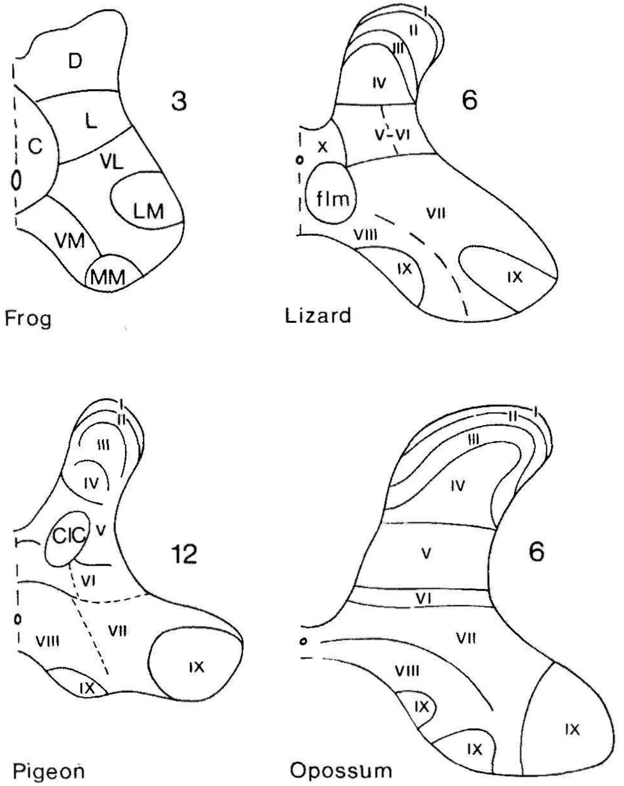


Fig. 11 Diagrams indicating the parcellation of the spinal gray in the frog *Rana catesbeiana* (based on Ebbesson, 1976a), the lizard *Tupinambis nigropunctatus* (the present study), the pigeon *Columba livia* (after Leonard and Cohen, 1975a) and the opossum *Didelphis virginiana* (after Martin and Fischer, 1968). Abbreviations: C, central field; ClC, Clarke's column; D, dorsal field; flm, fasciculus longitudinalis medialis; L, lateral field; LM, lateral motor field; MM, medial motor field; VL, ventrolateral field; VM, ventromedial field.

Columba livia (Leonard and Cohen, 1975a) and the opossum *Didelphis virginiana* (Martin and Fischer, 1968). In the frog the cell groups in the spinal cord show a poor differentiation. Therefore, the frog spinal gray has been subdivided by Ebbesson (1976a) into a number of fields, coinciding with afferent fiber systems, rather than into laminae. Throughout all amniotes discussed (Fig. 11) a strong resemblance exists in the organization of the spinal gray, particularly in the dorsal horn. It appears that Rexed's parcellation of the spinal gray can be applied to all amniotes studied so far. It should be emphasized, however, that in a detailed layer by layer comparison connectional data should also be taken into consideration. In this context it should be noted that in terrestrial vertebrates the pathways descending from the brain stem to the spinal cord terminate in comparable areas of the spinal gray (ten Donkelaar, 1976b). Similarities in the pattern of distribution of the dorsal root as well as some data on the cells of origin of non-primary ascending pathways and propriospinal fibers will be discussed in Chapters VII to IX.

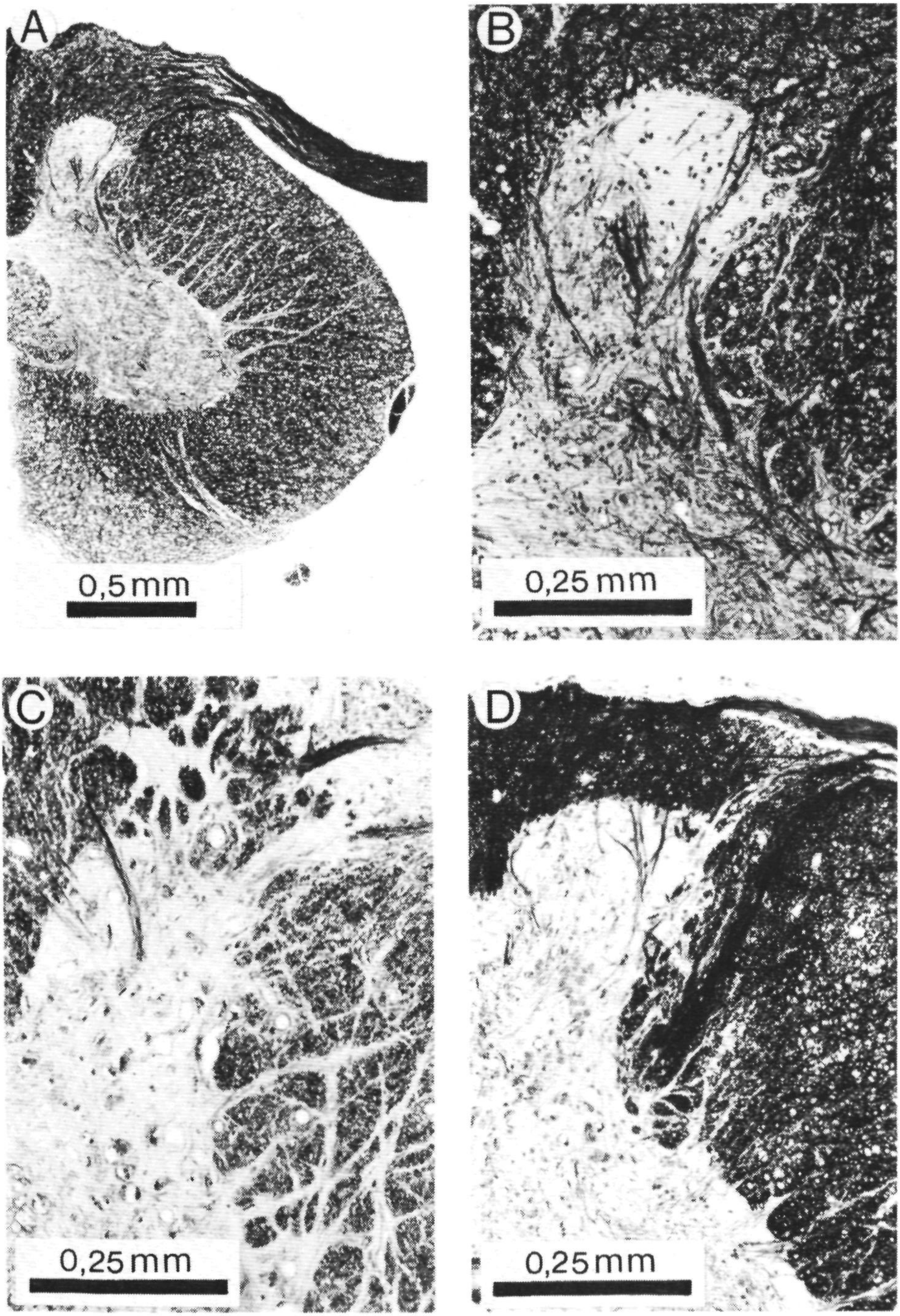
VI FIBER PATTERN IN THE SPINAL CORD

In this chapter a preliminary reconnaissance of the fiber pattern in the spinal cord of the three reptilian species studied will be presented, based on normal Häggqvist and Klüver-Barrera stained material. The following systems will be discussed: A) the dorsal root and its distribution into the spinal cord, and B) the fiber systems in the lateral and ventral funiculi.

A The distribution of dorsal root fibers into the spinal cord

It has been reported that in reptiles, as in mammals, each dorsal root can be divided into a large-fibered medial and a thin-fibered lateral bundle (Ariëns Kappers et al., 1936). The large diameter dorsal root fibers bifurcate and send ascending and descending branches into the dorsal funiculus. The smaller fibers, which also bifurcate, contribute their branches to the dorsolateral fascicle, a bundle also known as Lissauer's tract. However, since Lissauer's 1885 description of small myelinated axons from the dorsal root entering the dorsolateral fascicle, the organization of the dorsal rootlets into medial and lateral divisions has been the subject of considerable controversy (cf. R. Snyder, 1977, for discussion). Snyder's results indicate that a 'lateral division' of the dorsal root is present in the monkey, but not in the cat. The tract of Lissauer receives, in addition to small caliber dorsal root fibers, axons from dorsal horn neurons (LaMotte, 1977).

In reptiles some variation has been reported in relation to the course of the dorsal root fibers after their entrance into the cord. In turtles (Banchi, 1903; de Lange, 1917) and in snakes (Cajal, 1891; Retzius, 1894, 1898; van Gehuchten, 1897; de Lange, 1917) a projection of dorsal root fibers to the lateral funiculus has been claimed.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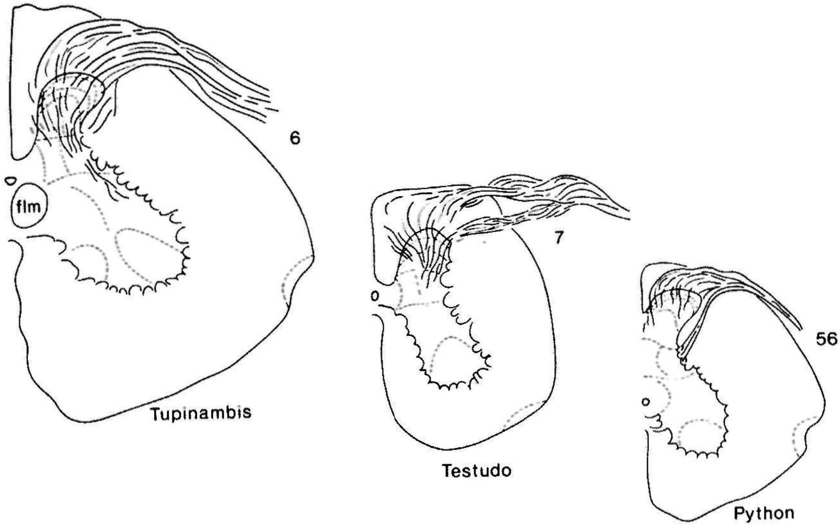


Fig. 13 Diagrammatic representation of the distribution of dorsal root fibers into the spinal cord based on Klüver-Barrera material in *Tupinambis nigropunctatus*, *Testudo hermanni* and *Python reticulatus*.

Analysis of Klüver-Barrera material (Figs. 12, 13) showed that in *Tupinambis nigropunctatus*, the dorsal root enters the spinal cord over a broad zone, whereas in *Testudo hermanni* and *Python reticulatus*, the dorsal root splits up into a dorsal and a ventral bundle, the latter passing through the lateral funiculus. In the turtle *Testudo hermanni* the ventral bundle mentioned above enters the spinal gray via the zone of Lissauer, whereas in *Python reticulatus* this distinct bundle of medium-sized fibers (3 - 6 μm axon diameter) courses through the dorsal part of the lateral funiculus to the lateral border of area V - VI (Figs. 12D,

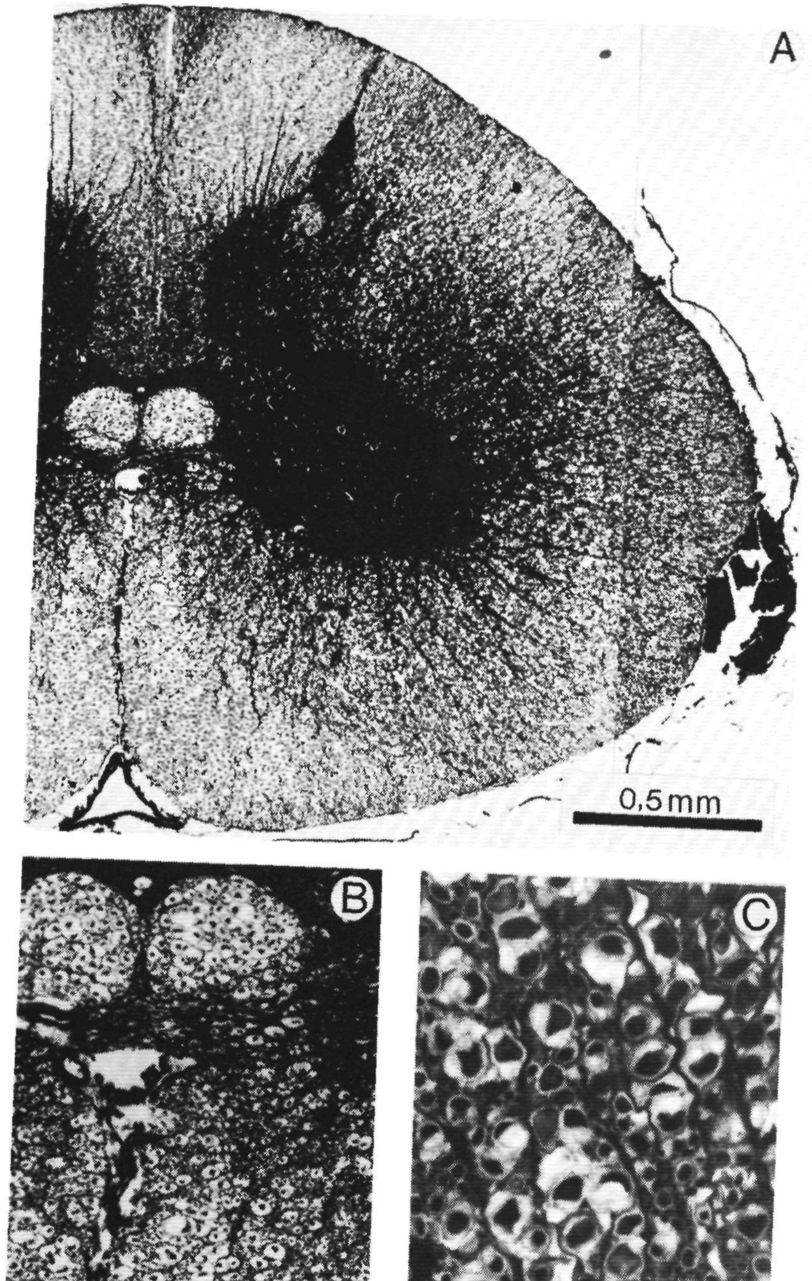
Fig. 12 Photomicrographs of Klüver-Barrera material showing the pattern of fiber entry into the dorsal horn. A, B, *Tupinambis nigropunctatus* (6th segment); C, *Testudo hermanni* (7th segment); D, *Python reticulatus* (56th segment).

13). The distinction made between medium-sized and small fibers as mentioned in the earlier literature (cf. Ariëns Kappers et al., 1936) is only ill-defined: the various types of fibers are mostly intermingled. Dorsal root fibers pass not only to the spinal gray but some enter the dorsal funiculus.

To describe the fiber pattern of the various systems in the spinal cord (see also fig. 14) the general terms small, medium-sized and coarse fibers are used. These terms are not strictly defined, but in general it may be said that the axon diameter of the small fibers is 3 µm or less, whereas that of the medium-sized ranges from 3 - 6 µm, and that of the coarse fibers from 6 - 12 µm.

The dorsal funiculus as seen in Häggqvist-stained material is composed of various fiber systems (Figs. 15 - 17). In the lizard *Tupinambis nigropunctatus* a zone of medium-sized fibers and a few coarse fibers is found at variable positions. Just rostral to the cervical intumescence as well as caudal to the lumbosacral intumescence these fibers lie dorsolaterally, whereas at levels between the intumescences this bundle occupies a ventrolateral position. In the intumescences, these fibers take a characteristic position in a more or less horizontal region of the dorsal funiculus. It seems likely that this bundle of coarse and medium-sized fibers is constituted by the bifurcating 'medial division' fibers of the dorsal root. Since long ascending fibers from lumbar cord levels are situated dorsomedially in the dorsal funiculus of the cervical cord, an area where only small fibers are found, it seems likely that the coarse and medium-sized fibers mentioned above, become thinner as they ascend. This phenomenon might be responsible for the absence of frontal accumulation in the dorsal funiculus of *Tupinambis nigropunctatus* (cf. Chapter IV).

Fig. 14 Photomicrographs of Häggqvist sections. A, 6th spinal segment of *Tupinambis nigropunctatus*; B, idem, detail of the ventral funiculus; C, detail of the ventral funiculus in *Testudo hermanni*, showing in particular the axons and their myelin sheaths (7th segment).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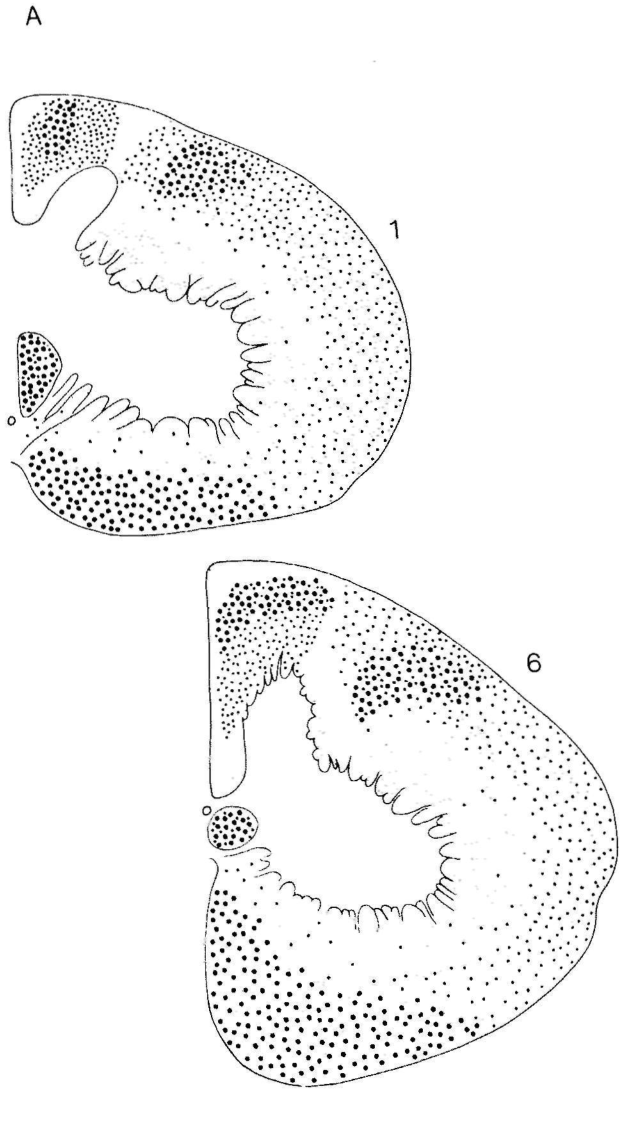
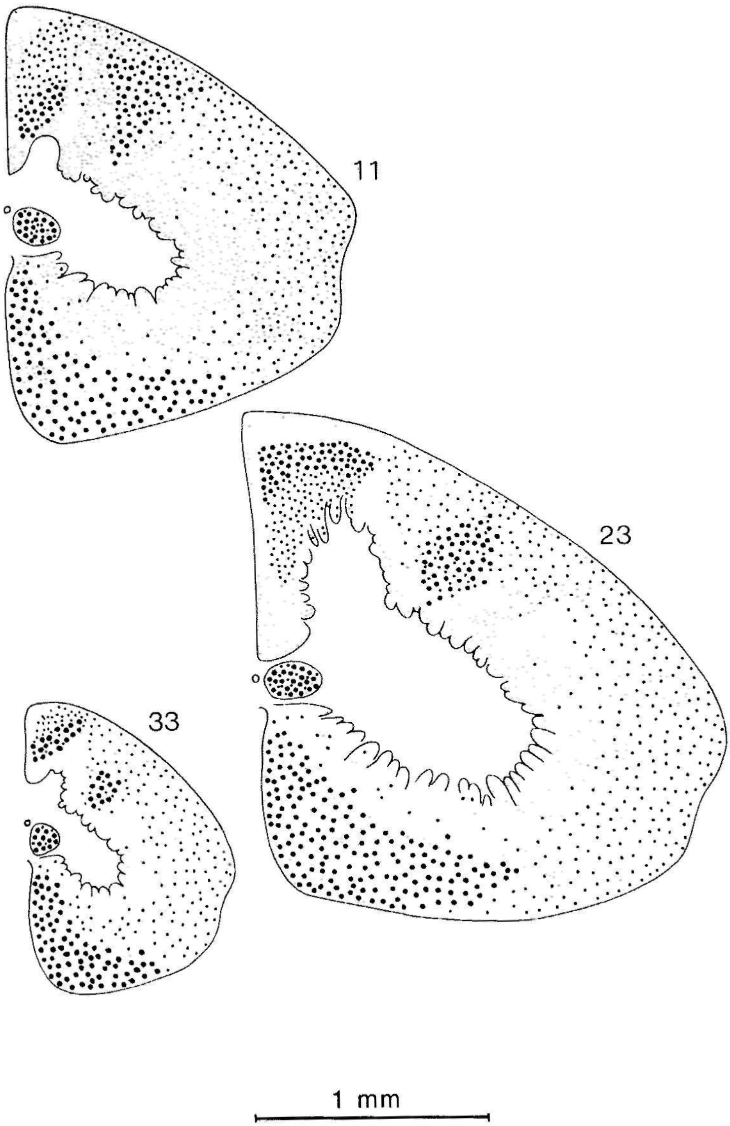


Fig. 15A, B Diagrammatic representations of transverse sections through representative levels of the spinal cord in the lizard *Tupinambis nigropunctatus*, showing the fiber pattern, based on Häggqvist material. Dots of three different sizes are employed to indicate small (3 µm or less), medium-sized (3 - 6 µm) and coarse (6 - 12 µm in axon diameter) fibers. In order to avoid crowding fibers less than one micron in diameter have not been indicated in these diagrams.

B



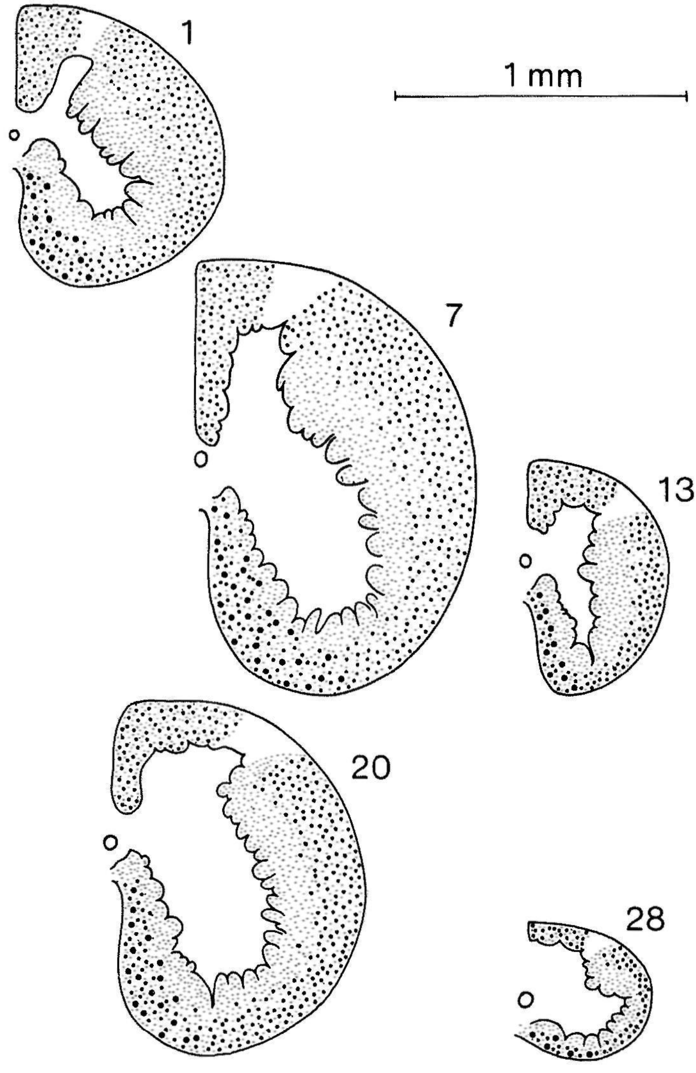


Fig. 16 Diagrammatic representations of transverse sections through representative levels of the spinal cord in the turtle *Testudo hermanni*, showing the fiber pattern based on Häggqvist material. For code see figure 14.

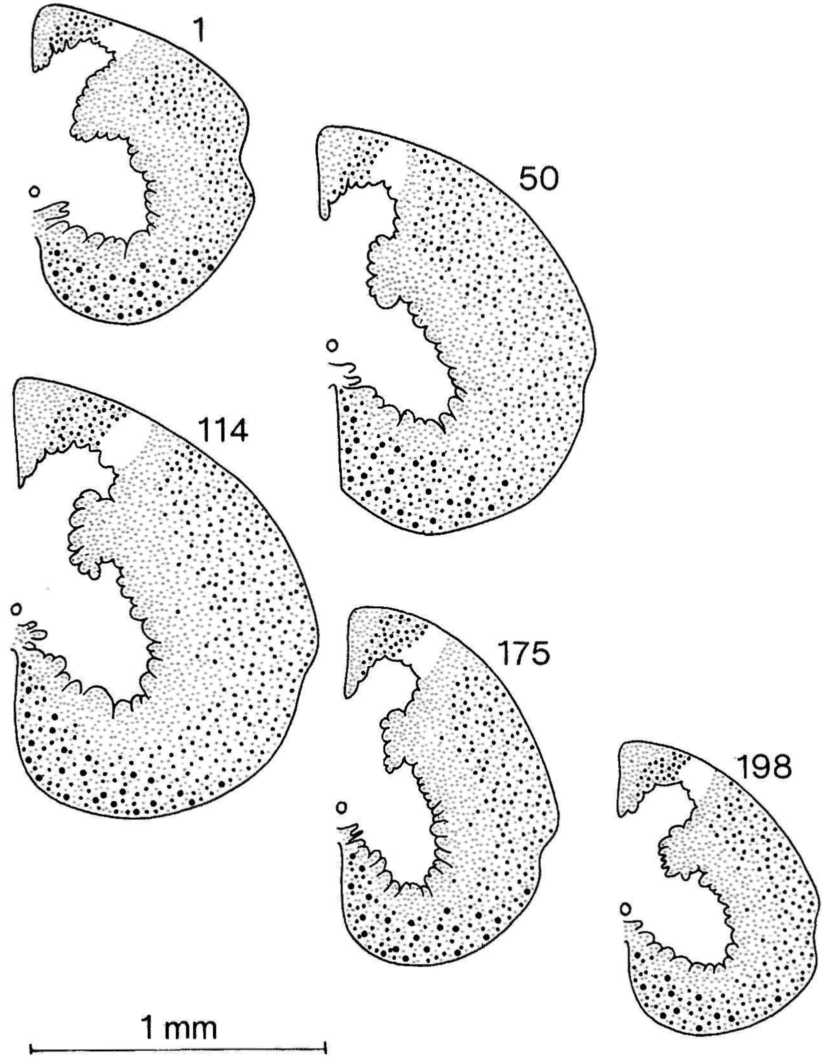


Fig. 17 Diagrammatic representations of transverse sections through representative levels of the spinal cord in the snake *Python reticulatus*, showing the fiber pattern based on Häggqvist material. For code see figure 14.

The remaining part of the dorsal funiculus is composed of small fibers (2 - 3 um axon diameter). In the region between the dorsal and the lateral funiculi, where the dorsal roots enter the spinal cord, an area of very small fibers (less than one micron axon diameter) is easily recognized, i.e. the dorsolateral fasciculus or tract of Lissauer.

Coarse fibers are not present in the dorsal funiculus of the turtle *Testudo hermanni* and the snake *Python reticulatus*. In *Testudo hermanni* (Fig. 16), the dorsal funiculus is composed of intermingled small and medium-sized fibers, whereas in *Python reticulatus*, medium-sized fibers are more laterally situated (Fig. 17). The bulk of the dorsal funiculus in *Python reticulatus* is composed of small fibers.

B Fiber pattern in the lateral and ventral funiculi

In Häggqvist material (Figs. 14 - 17) two major zones can be distinguished in the lateral as well as in the ventral funiculus: 1) a superficial zone, which in the ventral funiculus is composed of mainly coarse fibers, whereas in the lateral funiculus all types of fibers (small, medium-sized and coarse) are found; 2) an inner zone, surrounding the spinal gray, which consists predominantly of small fibers. As will be detailed further this zone contains propriospinal fibers.

It should be noted that the largest sizes only occur in the lizard *Tupinambis nigropunctatus*. In *Testudo hermanni* and *Python reticulatus*, the axon diameters of the coarse fibers do not extend beyond 7 µm. It must be emphasized that the position of the different tracts as well as the sizes of the areas they occupy, may change during their course through the spinal cord. Therefore, the structure of the cord at various levels may differ considerably. It is obvious that the cord cannot be studied without taking these changes into consideration and thus, the white matter throughout the whole extent of the cord has to be examined.

The fiber pattern present in the ventral and lateral funiculus will now be discussed.

1a. *The superficial zone of the lateral funiculus*

In the reptiles studied the superficial zone of the lateral funiculus is composed of intermingled, medium-sized and small fibers (Figs. 14 - 17). In *Tupinambis nigropunctatus*, the most dorsal part of the lateral funiculus contains in addition a conspicuous zone of closely packed medium-sized and coarse fibers (4 - 8 µm). This zone has been experimentally shown to represent the crossed rubrospinal tract (ten Donkelaar, 1976a, b; ten Donkelaar and Nieuwenhuys, 1979). A rubrospinal pathway has also been demonstrated in the lizards *Lacerta viridis* (Robinson, 1969) and *Lacerta galloti* (ten Donkelaar and de Boer - van Huizen, 1978a), and in *Testudo hermanni* (ten Donkelaar, 1976a, b). In Häggqvist material of this turtle, rubrospinal fibers could not be identified as a separate entity. It is important to note that in *Python reticulatus* with experimental techniques (ten Donkelaar, 1976a, b) no evidence for the presence of a rubrospinal tract has been found. Other descending fibers in the lateral funiculus originate in the nucleus reticularis inferior and in the nucleus raphes inferior (Robinson, 1969; ten Donkelaar, 1976a, b; Cruce, 1975, 1979; ten Donkelaar and de Boer - van Huizen, 1978a).

The large remainder of the superficial zone of the lateral funiculus consists mainly of long ascending fibers projecting to the brain stem and diencephalon. Investigators working with normal material have described spinocerebellar, spinobulbar and spinotectal components (Ariëns Kappers et al., 1936). The latter two components have been designated by Ebbesson (1967) as the lemniscus spinalis, a term which was introduced by Herrick (1914, 1930, 1948) in his extensive studies of the urodelan brain. Degeneration studies following hemicordotomies (Ebbesson, 1967, 1969; Pedersen, 1973) have revealed that the spinal lemniscus can be divided into: a) spinorhombencephalic, b) spinomesencephalic and c) spinothalamic projections.

In the spinal cord and even at the level of the spinobulbar junction the fibers, which ascend towards the brain stem constitute a continuous superficial fiber zone (cf. also ten Donkelaar and Nieuwenhuys, 1979). This system comprises: a) a large spino-reticular component which terminates mainly in the caudal part of the ipsilateral reticular formation; b) spinocerebellar fibers which at caudal sections of the brain stem cannot be separated from the spinal lemniscus, but more rostrally can be delimited as distinct dorsal and ventral spinocerebellar tracts; and c) a small spinomesencephalic and spinothalamic projection.

1b. *The superficial zone of the ventral funiculus*

This zone contains the bulk of the descending fibers from the brain stem to the spinal cord (ten Donkelaar and Nieuwenhuys, 1979). It is characterized by the presence of many coarse fibers (6 - 10 μm axon diameter) intermingled with some smaller fibers. In *Tupinambis nigropunctatus* (Fig. 15) a number of very coarse fibers (8 - 12 μm axon diameter) is separated off from the main part of the ventral funiculus by an accessory commissure. This portion of the ventral funiculus, lying dorsal to that commissure, contains interstitiospinal fibers (ten Donkelaar, 1976b) which originate in the nucleus interstitialis of the fasciculus longitudinalis medialis. Since such an accessory commissure is indistinct in *Testudo hermanni* and *Python reticulatus* a separate zone of interstitiospinal fibers cannot be distinguished in Häggqvist material of these species.

The main part of the superficial zone of the ventral funiculus is composed of reticulospinal and vestibulospinal fibers (Robinson, 1969; ten Donkelaar, 1976a, b; ten Donkelaar and Nieuwenhuys, 1979). In normal material no clear distinction between these two fiber contingents can be made (Figs. 15 - 17). With anterograde degeneration techniques, however, it has been shown that vestibulospinal fibers constitute the most peripheral rim, the reticulospinal fibers lying lateral to the vestibulo-spinal fibers (ten Donkelaar, 1976b).

2. *The inner zone of the lateral and ventral funiculi*

The inner zone of the lateral and ventral funiculi is predominantly composed of small-sized fibers (Figs. 15 - 17). These parts of the funiculi bordering the gray matter contain mainly propriospinal fibers, arising from interneurons. The connections of these interneurons with the motoneurons are especially important since spinal motoneurons are only to a small extent directly affected by fibers conducting nerve impulses from the periphery or from the brain. Most influences on motoneurons are exerted via spinal interneurons (Gelfan et al., 1974). Short propriospinal fibers are located in the deepest parts of the funiculi, whereas long propriospinal fibers are located more peripherally (van Beusekom, 1955, cat; and chapter VIII).

Conclusions:

The analysis of Häggqvist and Klüver-Barrera material in the reptiles studied allows the following conclusions:
1) At the site of entrance of the dorsal root into the spinal cord no clear segregation of large fibers medially and smaller fibers laterally has been observed. A peculiarity for reptiles seems to be a lateral bundle of medium-sized primary afferent fibers which traverses the dorsal part of the lateral funiculus.
2) The dorsal funiculus is composed of various fiber systems. The possibility has been considered that coarse and medium-sized fibers (as defined in the present study) which are derived from the dorsal root become thinner as they ascend. This phenomenon might be responsible for the absence of frontal accumulation in the dorsal funiculus of *Tupinambis nigropunctatus*.
3) In the lateral and ventral funiculi two major zones can be distinguished in Häggqvist material: a) a superficial zone which in the ventral funiculus is composed mainly of coarse fibers, whereas in the lateral funiculus all types of fibers are found; and b) an inner zone, surrounding the spinal gray which consists mainly of small fibers.

4) In the superficial zone of the lateral and ventral funiculi several descending pathways could be delineated, which stand out conspicuously in the spinal cord because of their high contingent of coarse fibers. The large remainder of the superficial zone of the lateral funiculus consists mainly of long ascending fibers projecting to the brain stem and diencephalon.
5) The inner zone of the lateral and ventral funiculi is predominantly composed of small-sized fibers. These parts of the funiculi bordering the gray matter contain mainly propriospinal fibers, arising from interneurons (see Ch. VIII).

The study of normal material as presented in the present and previous chapters has furnished the basis for the experimental investigations to be described in the following chapters.

Introduction

In this chapter the results of dorsal root transections in 12 lizards (*Tupinambis nigropunctatus*), 13 turtles (*Testudo hermanni*) and 4 snakes (*Python reticulatus*) will be described. Anterograde degeneration techniques (Nauta and Gygax, 1954; Fink and Heimer, 1967) have been used in the above-mentioned reptiles to study the distribution of primary afferent fibers within the spinal cord as well as to the brain stem. In addition, a modification of the horseradish peroxidase (HRP) technique has been used. In 8 turtles (6 *Pseudemys scripta elegans* and 2 *Testudo hermanni*) the enzyme HRP has been applicated to the proximal stump of the transected dorsal root (modified after Proshansky and Egger, 1977).

A Anterograde degeneration experiments

Results

1) Dorsal root projections in the lizard *Tupinambis nigropunctatus*.

The distribution pattern of the dorsal root fibers will first be described for the lizard *Tupinambis nigropunctatus*. Figure 18 shows schematic drawings of the rostrocaudal distribution of degeneration following dorsal root transections at some representative spinal cord segments, viz., the lumbar intumescence (the 24th segment), the thoracic cord (the 15th segment) and the cervical intumescence (the 8th spinal segment). Because of similarity in projection pattern of dorsal root fibers entering the spinal cord at different levels, the following observations are presented as a general description of this pattern with level-dependent differences inserted where appropriate to qualify the general description.

The distribution of degenerating fibers following a dorsal root transection remains restricted to the side of the lesion.

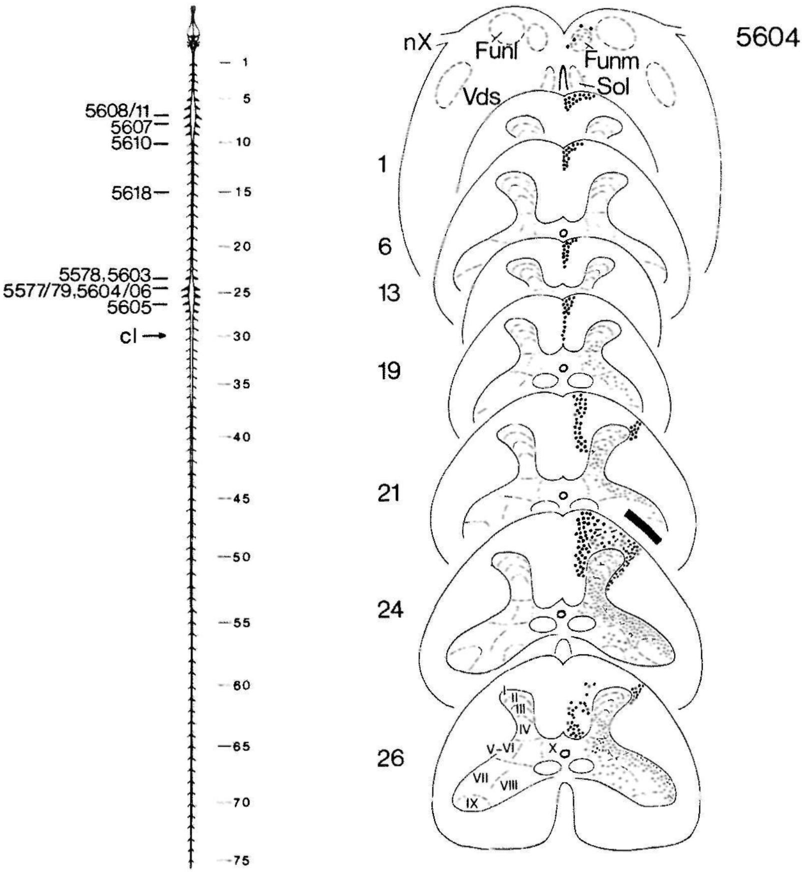


Fig. 18A Dorsal root transections in the lizard *Tupinambis nigropunctatus*, at the left the levels of the dorsal rhizotomies carried out, at the right a semidiagrammatic representation of the rostrocaudal distribution of degeneration following a lumbar (segment 24) dorsal root transection. In this figure as well as in the figures 18B - 20 and 23 coarse dots and broken lines indicate transversely respectively longitudinally cut degenerating fibers, whereas small dots represent evidence of preterminal degeneration. In addition, the spinal gray areas are also indicated by broken lines. Abbreviations: cl, level of cloaca; Funl, nucleus funiculi dorsalis pars lateralis; Funm, nucleus funiculi dorsalis pars medialis; n X, nervus vagus; Sol, nucleus tractus solitarii; V ds, nucleus descendens nervi trigemini.

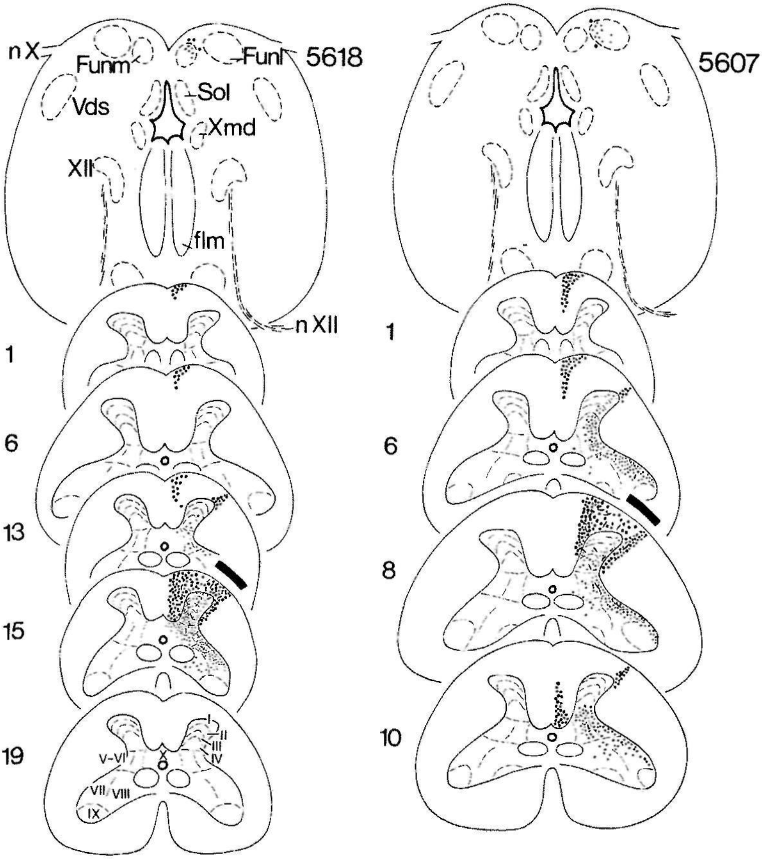


Fig. 18B Semidiagrammatic representation of the rostrocaudal distribution of degeneration following a thoracic (segment 15, at the left) and a cervical (segment 8, at the right) dorsal root transection in the lizard *Tupinambis nigropunctatus*. For symbols cf. Fig. 18A. Abbreviations: flm, fasciculus longitudinalis medialis; Funl, nucleus funiculi dorsalis pars lateralis; Funm, nucleus funiculi dorsalis pars medialis; n X, nervus vagus; n XII, nervus hypoglossus; Sol, nucleus tractus solitarii; V ds, nucleus descendens nervi trigemini; X md, nucleus motorius dorsalis nervi vagi; XII, nucleus nervi hypoglossi.

a) *Dorsal root entry zone*: In *Tupinambis nigropunctatus*, the dorsal root enters the spinal cord over a broad zone. At the site of entrance in all segments studied no clear segregation of medium-sized fibers medially and smaller fibers laterally was observed. These fibers intermingle with each other throughout their course. The subdivision into contingents of medium-sized fibers medially and smaller fibers laterally is further obscured by the presence of a more or less prominent bundle of degenerating medium-sized fibers passing via the most dorsal part of the lateral funiculus (Fig. 18).

b) *Rostrocaudal distribution*: The total longitudinal projection of single dorsal roots to the spinal cord in *Tupinambis nigropunctatus* varies considerably. In this lizard the rostrocaudal extent of the distribution of degenerating fibers within the spinal gray ranges from two to seven segments. A progressive diminution in the proportion of the dorsal funiculus occupied by degenerating fibers (cf. Fig. 18) and in the distribution to the spinal gray was noted in passing rostrally as well as caudally. The most restricted projection was observed following thoracic dorsal root transections, whereas the most extensive longitudinal projection was noted following lumbar dorsal rhizotomies. A certain proportion of the primary afferent fibers in the dorsal root enters the dorsal funiculus and thence passes rostrally to reach the dorsal funicular nuclei.

Degenerating fibers in the zone of Lissauer ascend and descend over only two or three segments. The longitudinal extent of degeneration in the dorsal part of the lateral funiculus was restricted to the segment of the lesion.

c) *Segment of entry*: Within the rhizotomized spinal segments, degenerating fibers and their endings were found in every area of the ipsilateral spinal gray distinguished in the lizard *Tupinambis nigropunctatus* (Fig. 18). In area I only a few terminals were present, probably derived from the zone of Lissauer. Degenerating fibers could be traced from the zone of Lissauer to the most dorsal area of the lizard spinal gray, running perpendicular to the longitudinal axis of the cord.

Area II was penetrated by bundles of degenerating fibers on their way to area III. Only a small amount of preterminal debris was found in area II.

In all segments studied, areas III and IV received a relatively dense projection of primary afferents. Fibers entered area III either via area II or directly from the dorsal funiculus, whereas degenerating fibers reached area IV mainly directly from the dorsal funiculus. Degenerating fibers to the base of the dorsal horn (area V - VI) and to more ventral parts of the spinal gray entered these areas mainly via area IV, particularly from the dorsal funiculus. In addition, some fibers passed from the dorsal root through the most dorsomedial part of the lateral funiculus, traversed areas III and IV to terminate in the lateral part of area V - VI as well as in more ventrally situated areas. In the intumescences, preterminal and terminal degeneration in area V - VI was found mainly laterally. At thoracic levels, however, a more or less distinct concentration of preterminal degeneration was noted medially (Fig. 18).

Two bundles of degenerating fibers traversed area V - VI to cascade down into the ventral horn: a bundle of degenerating fibers from the dorsal funiculus and a second one passing along the lateral border of the spinal gray (Fig. 18). Particularly the lateral part of area VII - VIII received degenerating fibers. The ventromedial part of area VII - VIII, however, received only a slight projection from the dorsal root. At the level of the intumescences the most ventral site of termination of the primary afferents of the spinal cord observed was the dorsolateral part of the lateral motoneuron column (area IX). Preterminal degeneration was present at only a short distance of the perikarya of these motoneurons. Apart from this projection to area IX the ventral horn gray matter was practically devoid of degenerating fragments.

The centrally situated area X was uniformly only lightly populated with degenerating elements. In all instances, no crossing fibers could be observed.

In summary, within the rhizotomized spinal segment degenerating fibers and their endings were found particularly in area III and area IV, less in area I and only very sparsely in area II. In the thoracic cord, a concentration of preterminal degeneration was found in the medial part of areas IV to VI. The ventrolateral bundle of medium-sized fibers, passing via the most dorsal part of the lateral funiculus, entered the spinal gray at the lateral side of areas IV to VI. Together with primary afferent fibers passing through the dorsal funiculus degenerating fibers of this ventrolateral bundle were distributed to the dorsolateral part of area VII - VIII as well as to the dorsolateral part of the lateral motoneuron column.

d) *Primary afferents of the dorsal root projecting to the brain stem*: A certain proportion of the primary afferent fibers of the dorsal root which entered the dorsal funiculus, were found to pass rostrally to reach the dorsal funicular nuclei. Following dorsal root transections in the lumbar intumescence (e.g. segment 24, Fig. 18A) degenerating fibers could be traced via the most medial part of the dorsal funiculus to terminate in the medial part of the nucleus funiculi dorsalis pars medialis (Funm, Fig. 18A). A lesion of the 15th (thoracic) dorsal root resulted in degenerating fibers in a more lateral position in the dorsal funiculus and terminal degeneration in the lateral part of the nucleus funiculi dorsalis pars medialis. Following dorsal root transections at the level of the cervical intumescence (e.g. segment 8, Fig. 18B) degenerating fibers were found in the dorsal funiculus in a still more lateral position as compared to thoracic lesions, whereas preterminal and terminal degeneration was present in the nucleus funiculi dorsalis pars lateralis (Funl, Fig. 18B). Therefore, these long ascending fibers show a gross somatotopical arrangement in such a fashion that fibers of caudal origin are most medial and those joining at more rostral levels are situated more laterally.

No evidence for a projection of primary afferents of the spinal cord to other brain stem nuclei than the dorsal funicular nuclei could be demonstrated.

2) Dorsal root projections in the turtle *Testudo hermanni*.

Figure 19 shows schematic drawings of the rostrocaudal distribution of degeneration following dorsal root transections at some representative spinal cord segments in the turtle *Testudo hermanni*, viz., just rostral to the lumbar intumescence (the 16th segment), the thoracic cord (the 10th segment) and the cervical intumescence (the 8th segment). The distribution of degenerating fibers following a dorsal root transection remains restricted to the side of the lesion.

a) *Dorsal root entry zone*: In *Testudo hermanni*, the dorsal root was found to split up into a dorsal and a ventral bundle, the latter entering the spinal gray via the zone of Lissauer (Fig. 19). As fibers of the dorsal rootlet approached the cord, no clear segregation of larger fibers medially and smaller fibers laterally was observed. Bundles of small fibers appeared to be randomly distributed among the larger fibers.

b) *Rostrocaudal distribution*: In the material studied degenerating fibers and terminals to the spinal gray could only be demonstrated in the segment of root entrance. Degenerating fibers in the zone of Lissauer as well as the longitudinal extent of degeneration in the most dorsal part of the lateral funiculus were also found to be restricted to the segment of the lesion.

c) *Segment of entry*: Within the rhizotomized spinal segment, degenerating fibers and their endings were found exclusively ipsilaterally. The apex of the dorsal horn, which occupies the area I - II, contained numerous degenerating small diameter fibers which were apparently derived from fibers passing via the dorsal funiculus. Degenerating fibers could be traced from the zone of Lissauer to area I - II, running perpendicular to the longitudinal axis of the cord.

The majority of the degenerating primary afferent fibers were found to terminate within the "neck" region of the dorsal

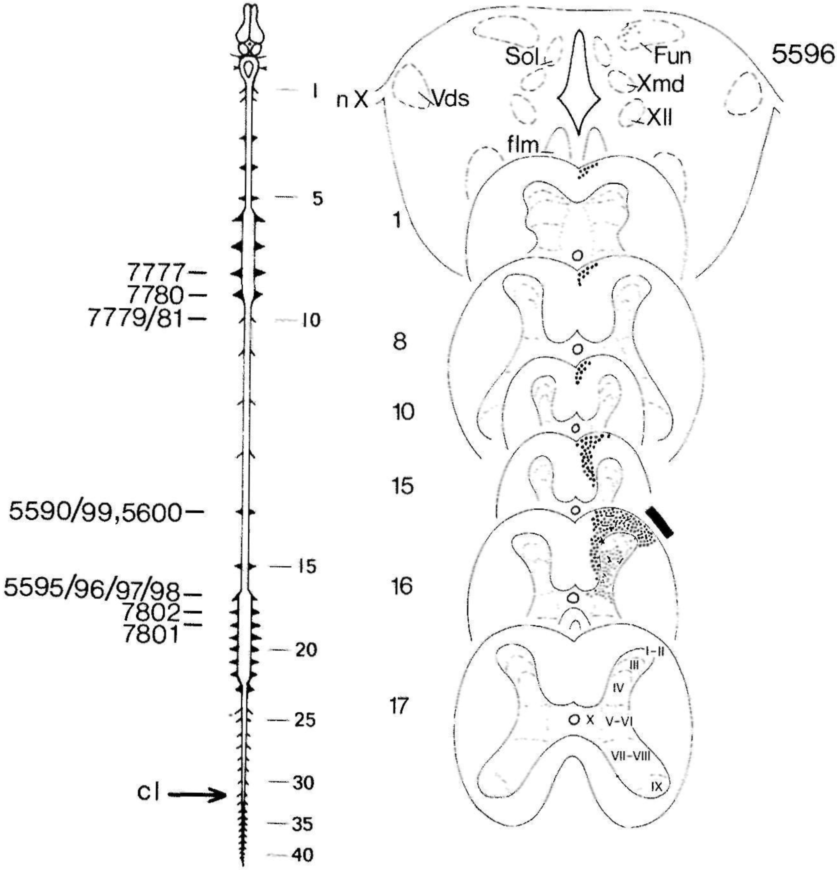


Fig. 19A Dorsal root transections in the turtle *Testudo hermanni*, at the left the levels of the dorsal rhizotomies carried out, at the right a semidiagrammatic representation of the rostrocaudal distribution of degeneration following a lumbar (segment 16) dorsal root transection. For symbols cf. Fig. 18A. Abbreviations: cl, level of cloaca; flm, fasciculus longitudinalis medialis; Fun, nucleus funiculi dorsalis; n X, nervus vagus; Sol, nucleus tractus solitarii; V ds, nucleus descendens nervi trigemini; X md, nucleus motorius dorsalis nervi vagi; XII, nucleus nervi hypoglossi.

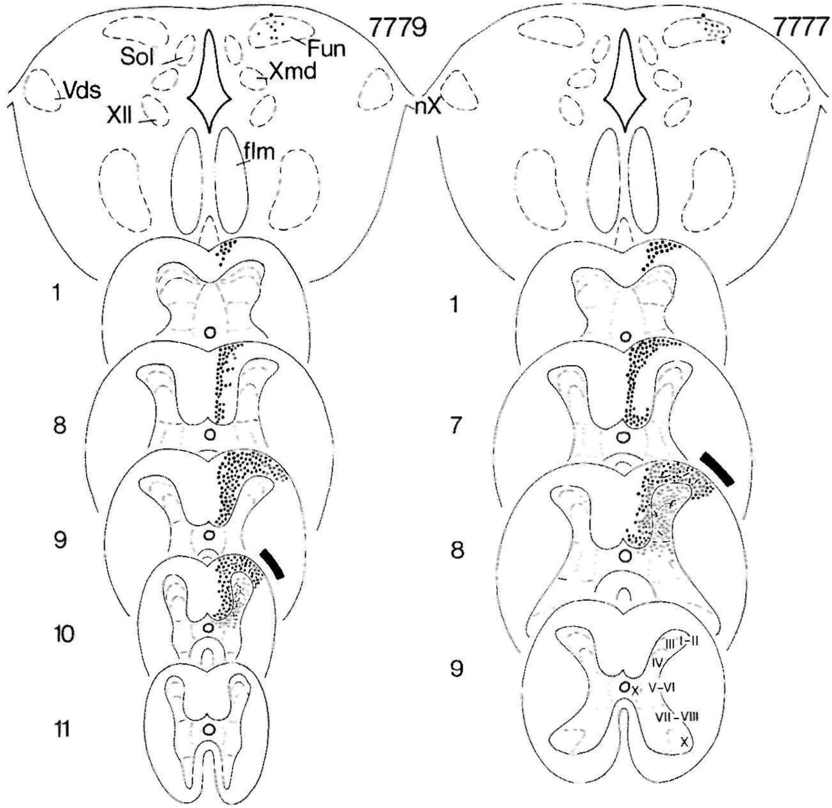


Fig. 19B Semidiagrammatic representation of the rostrocaudal distribution of degeneration following a thoracic (segment 10, at the left) and a cervical (segment 8, at the right) dorsal root transection in the turtle *Testudo hermanni*. For symbols cf. Fig. 18A. Abbreviations: flm, fasciculus longitudinalis medialis; Fun, nucleus funiculi dorsalis; n X, nervus vagus; Sol, nucleus tractus solitarii; V ds, nucleus descendens nervi trigemini; X md, nucleus motorius dorsalis nervi vagi; XII, nucleus nervi hypoglossi.

horn which consists of the areas III and IV. At thoracic levels, a more or less distinct concentration of terminal degeneration was found in the medial part of area IV, following thoracic dorsal rhizotomies.

The most ventral area of terminal degeneration was observed at the dorsal part of the area V - VI. The degenerating fibers were for the greater part derived from the dorsal funiculus, reaching this area by traversing both area III and area IV. In all instances no fibers were found to extend into area VII - VIII and area IX. Only few degenerating elements could be traced to the dorsal aspect of the centrally situated area X.

In summary, the distribution of degenerating fibers following dorsal root transections in the turtle *Testudo hermanni* was found to remain restricted to the side of the lesion. Within the rhizotomized spinal segment, degenerating fibers and their endings were found particularly in area III and area IV, less in area I - II. In the material studied the degenerating fibers in the spinal gray were observed only in the segment of root transection. Some fibers could be traced into the dorsal part of area V - VI and into the ipsilateral area X. However, no fibers were found to extend into the ventral horn.

d) *Primary afferents of the dorsal root projecting to the brain stem*: A small proportion of the primary afferent fibers of the dorsal root entered the dorsal funiculus and passed rostrally to reach the dorsal funicular nucleus. Following dorsal root transections in the lumbar intumescence (e.g. segment 16, Fig. 19A) degenerating fibers could be traced via the most medial part of the dorsal funiculus to terminate in the most medial part of the nucleus funiculi dorsalis (Fun, Fig. 19A). A lesion of the 10th (thoracic) dorsal root resulted in degenerating fibers in a more lateral position in the dorsal funiculus and terminal degeneration in the more lateral part of the nucleus funiculi dorsalis. Following dorsal root transections in the cervical intumescence (e.g. segment 8, Fig. 19B), degenerating fibers were found in the dorsal funiculus in a still more lateral position as compared to thoracic lesions, whereas preterminal and terminal degeneration was noted in the most lateral part of the nucleus funiculi dorsalis (Fig. 19B).

From a comparison of cases with lesions at various spinal levels (cf. Fig. 19), it is evident that the primary afferents of the dorsal root projecting to the brain stem are arranged in such a fashion that those of caudal origin are most medial and those joining at more rostral levels are situated more laterally. Although in *Testudo hermanni* on a cytoarchitectonic basis no division of the nucleus funiculi dorsalis into medial and lateral parts can be made (ten Donkelaar and Nieuwenhuys, 1979), the present experiments indicate that the projection of primary afferents from the spinal cord to this nucleus shows a clear somatotopical arrangement.

3) Dorsal root projections in the snake *Python reticulatus*.

Figure 20 illustrates schematic drawings of the rostrocaudal distribution of degeneration following dorsal root transections in the snake *Python reticulatus* at some representative spinal cord segments, viz., the 74th and the 25th spinal segments. The degenerating fibers following a dorsal rhizotomy were found exclusively ipsilateral to the transected dorsal root.

a) *Dorsal root entry zone*: In *Python reticulatus*, the distinction made between separate bundles of medium-sized and small fibers as reported for snakes in the earlier literature (cf. Ariëns Kappers et al., 1936), was only ill-defined; the various types of fibers were mostly found intermingled. At the site of entrance, the dorsal root splits up into a dorsal and a ventral bundle. The latter distinct bundle of medium-sized fibers coursed through the dorsal part of the lateral funiculus to the lateral border of the gray matter (Fig. 20).

b) *Rostrocaudal distribution*: In the material studied the total longitudinal projection of single dorsal roots to the spinal gray did not seem to extend beyond the adjacent segments. Degenerating fibers in the zone of Lissauer were found to ascend and descend for about two segments only. The longitudinal extent of degeneration in the dorsal part of the lateral funiculus remained restricted to the segment of the lesion.

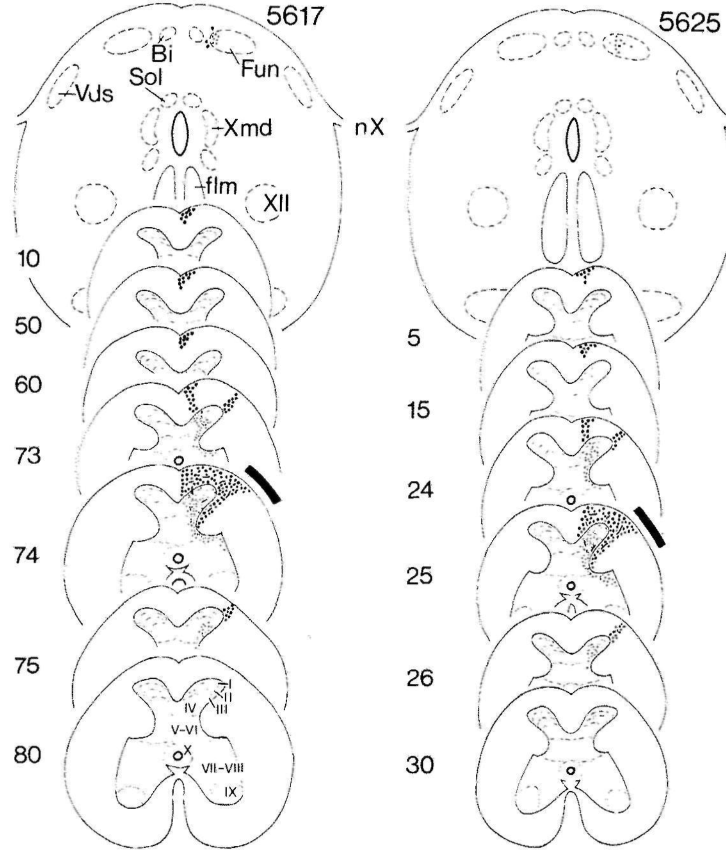


Fig. 20 Semidiagrammatic representation of the rostrocaudal distribution of degeneration following dorsal root transections in the snake *Python reticulatus* at the 74th (at the left) and the 25th (at the right) spinal segment. For symbols cf. Fig. 18A. Abbreviations: Bi, nucleus of Bischoff; flm, fasciculus longitudinalis medialis; Fun, nucleus funiculi dorsalis; n X, nervus vagus; Sol, nucleus tractus solitarii; V ds, nucleus descendens nervi trigemini; X md, nucleus motorius dorsalis nervi vagi; XII, nucleus nervi hypoglossi.

c) *Segment of entry*: Within the rhizotomized spinal segment, degenerating fibers and their endings were found exclusively ipsilateral to the side of the lesion. A bundle of degenerating dorsal root fibers curved through the dorsal funiculus and entered the dorsal horn from its dorsal and medial aspects. Degenerating fibers were distributed to area I, in which, however, only a few terminals were present. The degenerating fibers within the lateral region of area I were probably partly derived from the zone of Lissauer.

Area II was traversed by bundles of degenerating fibers on their way to area III and more ventrally situated areas. In area II only a very small amount of preterminal debris was observed. Many degenerating fibers from a single dorsal root were distributed to areas III and IV. Fibers entered these areas through or around the lateral aspect of area II, or by curving around the medial aspect of the dorsal horn in the dorsal funiculus.

Two bundles of degenerating fibers traversed area V - VI to terminate in the dorsolateral part of area VII - VIII. A bundle of degenerating dorsal root fibers passing via the dorsal funiculus, continued ventrally from area IV into areas V - VI and VII - VIII; and a second one passing along the dorsal part of the lateral funiculus, was found to enter the spinal gray at the lateral side of areas IV to V - VI. Preterminal debris within these areas appeared to be derived from both dorsal and ventral division fibers of the dorsal root mentioned above. However, no concentration of terminal degeneration was found in the medial part of these areas, as in the other reptiles studied.

The most ventral area of terminal degeneration was observed at the most dorsolateral part of area VII - VIII. Apart from this terminal degeneration, the ventral horn gray matter was otherwise practically devoid of degenerating debris.

In summary, within the rhizotomized spinal segment, degenerating fibers and their endings were found particularly in areas III and IV, while hardly any were distributed to areas I and II. No concentration of terminal degeneration was found in the medial parts of areas IV to V - VI. The ventrolateral bundle of medium-sized fibers, passing via the dorsal part of the lateral funiculus, entered the spinal gray at the lateral side of areas IV to V - VI. However, almost no fibers were found to extend into the ventral horn.

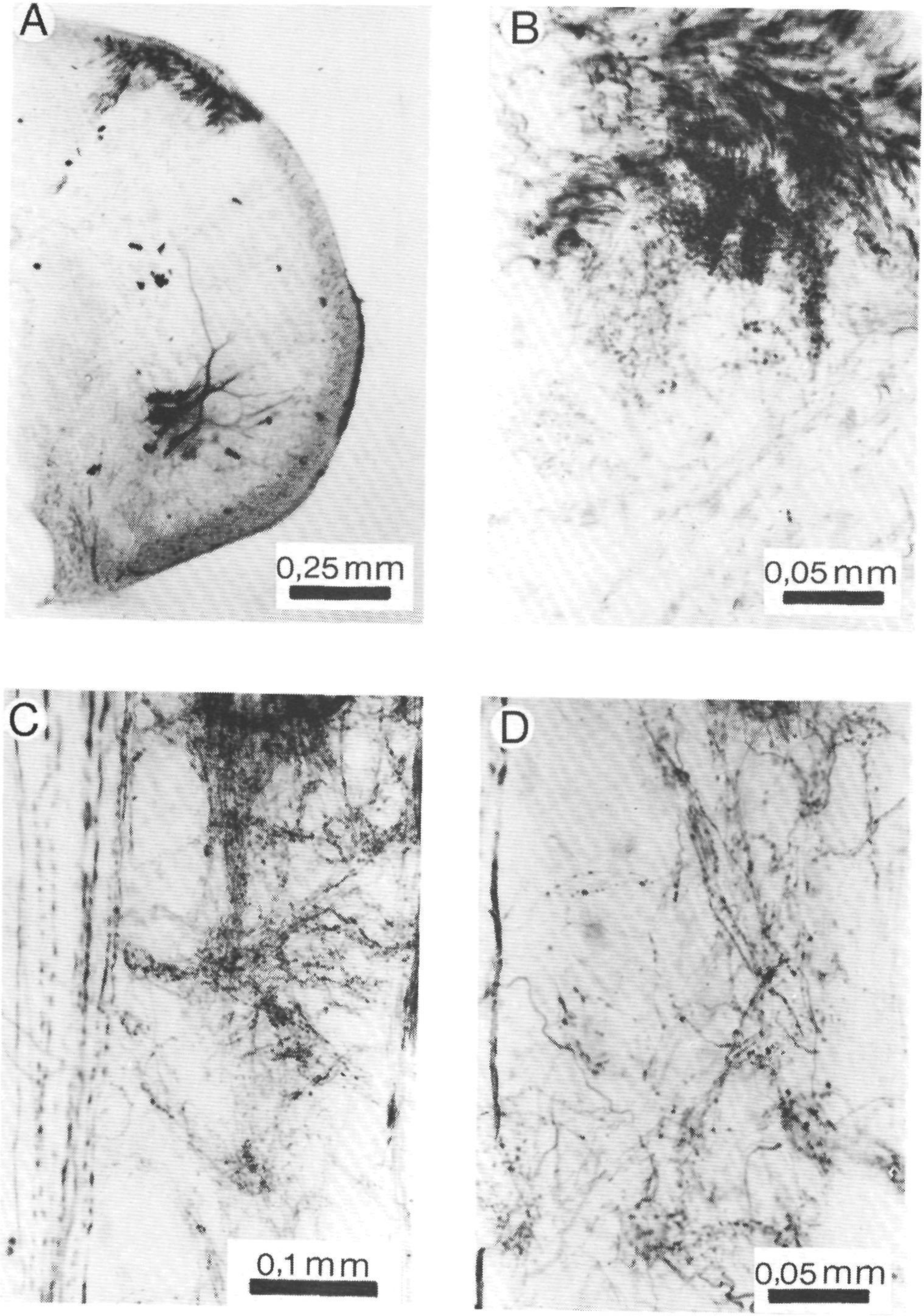
d) *Primary afferents of the dorsal root projecting to the brain stem*: To give a complete analysis of the fiber composition of the dorsal funiculus in the snake, the number of the lesions described in this chapter is much too small. However, a very small proportion of the primary afferent fibers of the dorsal root enters the dorsal funiculus and thence passes rostrally to reach the dorsal funicular nucleus. A gross somatotopical arrangement as observed in *Tupinambis nigropunctatus* and *Testudo hermanni* seems to be present also in *Python reticulatus* (Fig. 20).

B Staining of the dorsal root projection by anterograde movement of horseradish peroxidase

Notes on the technique employed

Anterograde movement of the enzyme horseradish peroxidase (HRP) within severed dorsal root fibers has been used to stain selectively primary afferent fibers to the spinal cord of the turtles *Pseudemys scripta elegans* and *Testudo hermanni*. The use of the enzyme HRP combines advantages of the Golgi technique with those of anterograde tracing techniques (Proshansky and Egger, 1977), since axonal detail is revealed, while the selective

Fig. 21 Photomicrographs of the 15th spinal segment of the turtle *Pseudemys scripta elegans*, illustrating the staining produced by anterograde movement of HRP. A, transverse section showing the staining of dorsal root fibers and in addition the retrogradely labeled motoneurons after damaging the ventral root; B, detail of the dorsal horn showing the pattern of filling of the primary afferent fibers, transverse section; C, oblique section (as indicated in Fig. 22) showing in part the terminal axonal arborizations within the dorsal horn and longitudinally oriented fibers in the dorsal funiculus; D, detail of the terminal axonal structures, oblique section.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application to a particular fiber system such as the dorsal root or the optic nerve (Scalia and Colman, 1974; Colman et al., 1976; Halpern et al., 1976) allows the clear identification of axonal origin. Although the stained fibers are severed from their cell bodies, axonal filling occurs before any of the morphological changes accompanying anterograde degeneration are observable (Scalia and Colman, 1974; Proshansky and Egger, 1977; Beattie et al., 1978). All intra-axonal reaction product was of the diffuse type. Such uniform staining, which is in contrast to the granular labeling associated with retrograde transport of HRP, is thought to result from damage to the cell membrane (LaVail, 1975; Hedreen and McGrath, 1977), allowing the enzyme to be distributed evenly throughout the entire cell. Single axons could be traced over considerable distance and morphological details such as fine collaterals and terminal arborizations were consistently observed. Terminal axonal structures occurred either as sequential arrays of swellings along a portion of the axon or as clusters formed by groups of single swellings on fine terminal stalks (Fig. 21). In the cat (Proshansky and Egger, 1977; Light and Perl, 1977; Brown and Fyffe, 1978), these structures were identified as axon terminals because they are identical to such structures described in Golgi studies (Beal and Fox, 1976; Beal and Cooper, 1978; Réthelyi, 1977; Réthelyi and Szentágothai, 1969). Recent electron microscopic observations of HRP-filled dorsal root fibers (Beattie et al., 1978) as well as of similar HRP-filled axonal structures, which are visualized following intracellular HRP-injections into spinal motoneurons (Cullheim and Kellerth, 1976, 1978; Cullheim et al., 1977), confirm them as typical presynaptic endings.

Since the pattern of filling of the primary afferent fibers, their collaterals as well as the terminal and "en passant" swellings in the turtles studied is directly comparable to that in the cat (Proshansky and Egger, 1977; Light and Perl, 1977), it seems likely that the swellings observed (cf. e.g. Fig. 21) represent axon terminals.

Results

In 8 turtles (6 *Pseudemys scripta elegans* and 2 *Testudo hermanni*) dry HRP has been applicated to the proximal stump of transected thoracic and lumbar dorsal roots (modified after Proshansky and Egger, 1977).

The proximal stump of the severed dorsal root and a discrete portion of the dorsal funiculus stained darkly, whereas a paler gold-brown coloration was visible in the dorsal horn.

a) *Dorsal root entry zone*: At the site of root entrance no clear segregation of medium-sized fibers medially and smaller fibers laterally could be observed. However, the darkly-stained area in the dorsal funiculus was composed of a massive number of medium-sized dorsal root fibers uniformly filled with HRP, whereas in Lissauer's tract thinner fibers were found labeled (Figs. 21A, B).

b) *Rostrocaudal distribution*: In the material studied the total longitudinal projection of single dorsal roots to the spinal gray remained restricted to the segment of the lesion. The longitudinal projection of fibers in the zone of Lissauer as well as in the most dorsal part of the lateral funiculus did not extend beyond the segment of root entrance.

c) *Segment of entry*: Within the spinal segments of the severed dorsal roots, terminal axonal arborizations were found in all areas of the dorsal horn. Abundant primary dorsal root terminations could be demonstrated within the ventral portion of area I - II. In both transversely and obliquely sectioned material (Figs. 21B, C, D, 22) it was evident that these terminals originate from dorsal root fibers which enter the spinal gray from the dorsal funiculus. The most dorsal portion of area I - II was almost entirely free from primary afferent endings. In addition to these endings formed by dorsal root fibers entering from the dorsal funiculus, thin primary afferent dorsal root fibers were also found to terminate in area I - II, particularly in its lateral portion. The terminal fields formed by these thin fibers were of two types: one consisted of longitudinally oriented

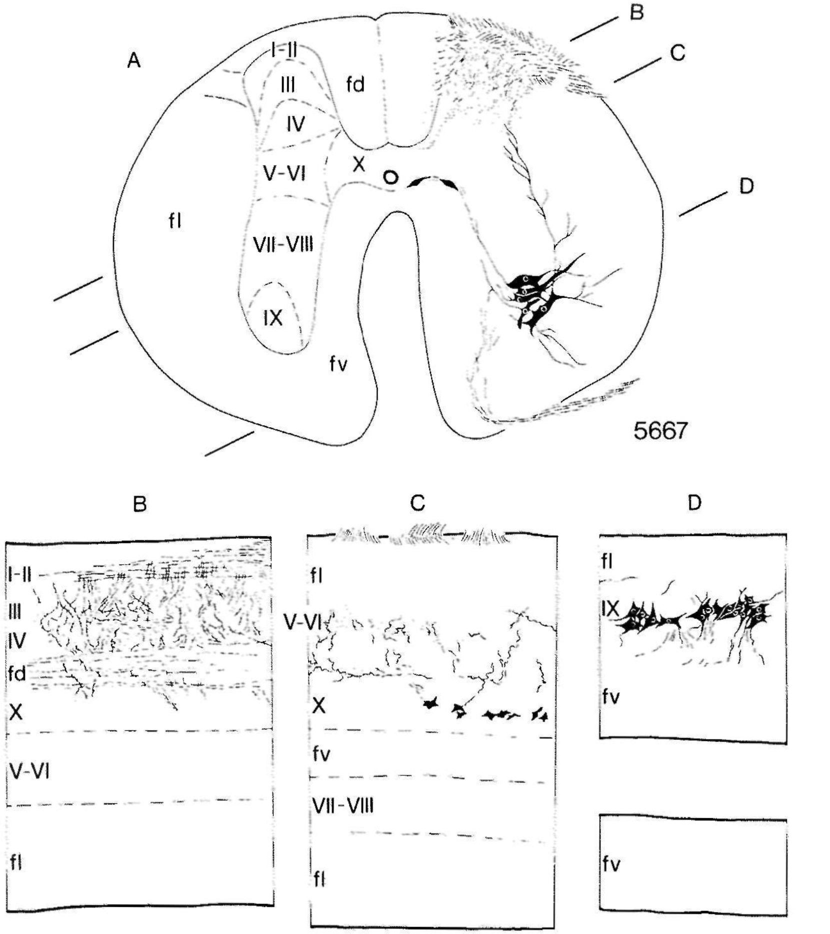


Fig. 22A Diagrammatic representation of the distribution of terminal axonal arborizations following HRP application to the cut dorsal root (15th spinal segment) in the turtle *Pseudemys scripta elegans*. The ventral root has also been damaged which results in the retrogradely labeling of motoneurons and small cells in the ventral border of area X. B, section showing the terminal axonal arborizations within the dorsal horn and in part the longitudinally oriented fibers in the dorsal funiculus; C, section showing in part the retrogradely labeled small cells in the ventral border of area X; D, section showing the retrogradely labeled motoneurons. Sections B, C and D have been indicated in diagram A. Abbreviations: fd, funiculus dorsalis; fl, funiculus lateralis; fv, funiculus ventralis. Roman numerals indicate the spinal gray areas outlined.

fibers which formed arrays of endings "en passant", and the other was composed of transversely oriented fibers with little rostro-caudal spread within the segment of root entrance. Both types are derived from dorsal root fibers running within Lissauer's tract. In areas III and IV terminals either as spherical dilatations on fine stalks or as sequential arrays of swellings along a portion of the axon (terminals "en passant"), were consistently observed. A more or less distinct concentration of terminals was observed in the medial part of area IV of the dorsal horn at thoracic levels.

The most ventrally situated terminal boutons were observed in the dorsal part of area V - VI. Besides, terminals "en passant" could be traced to the dorsal part of the centrally situated area X. The intermediate zone (area VII - VIII) and the ventral horn (inclusive area IX) were practically devoid of primary dorsal root terminals.

d) *Primary afferents of the dorsal root projecting to the brain stem*: In the material studied, labeled primary dorsal root fibers in the dorsal funiculus could only be followed through the directly adjacent segments, but not to the dorsal funicular nucleus.

The results obtained confirm the distribution pattern observed with anterograde degeneration techniques, however, primary afferent terminals could be demonstrated in the ventral portion of area I - II in more detail. In summary, the anterograde HRP-technique has been found to be an effective technique for demonstrating morphological detail of identified axons.

Commonly, ventral roots were also more or less damaged which resulted in retrograde transport of the enzyme HRP. In this way the presence of labeled motoneurons in the ventral horn (Figs. 21A, 22D) was observed. The visualization of motoneurons was comparable to that of the Golgi method. An extensive dendritic plexus was noted. Two dendrites were found particularly prominent, one extending medially and ventrally, the other extending dorsally along the medial border of the lateral funiculus. In thoracic segments, however, no retrogradely labeled cells could be observed in the ventral horn. Labeled terminal swellings of the dorsal root were observed in areas in which the distal part of the motoneuronal dendrites extend.

The most intriguing finding was the presence of retrogradely labeled small cells in the ventral border of the ipsilateral area X, at least, of the 13th to the 18th spinal segments (Fig. 22C). These cells may represent cells of origin of preganglionic autonomic fibers.

Discussion

In figure 23 the analysis of dorsal root transections in the lizard *Tupinambis nigropunctatus*, the turtle *Testudo hermanni* and the snake *Python reticulatus*, has been summarized. In all reptiles studied the distribution of degenerating fibers following a dorsal root transection remained restricted to the side of the lesion. At the site of root entrance no clear segregation of medium-sized fibers medially and smaller fibers laterally could be observed, a finding consistent with the observations in the normal material. The subdivision into contingents of medium-sized fibers medially and smaller ones laterally is further obscured by the more or less developed lateral bundle of medium-sized fibers, which passes through the lateral funiculus. The medium-sized fibers entering the dorsal funiculus bifurcate, send rostral branches as far as the medulla oblongata, and caudal branches downward for a variable number of segments. The smaller "lateral division" fibers enter the dorsolateral fasciculus directly and branch to ascend and descend for a few segments.

The rostrocaudal extent of the distribution of degenerating fibers within the spinal gray in the lizard *Tupinambis nigropunctatus* has been found to vary considerably. In the material studied, the most limited longitudinal projection of single dorsal roots to the spinal gray was observed in *Testudo hermanni* and *Python reticulatus*.

Within the rhizotomized spinal segments in *Tupinambis nigropunctatus*, degenerating terminals were present at only a short distance of the perikarya of motoneurons, whereas in *Testudo*

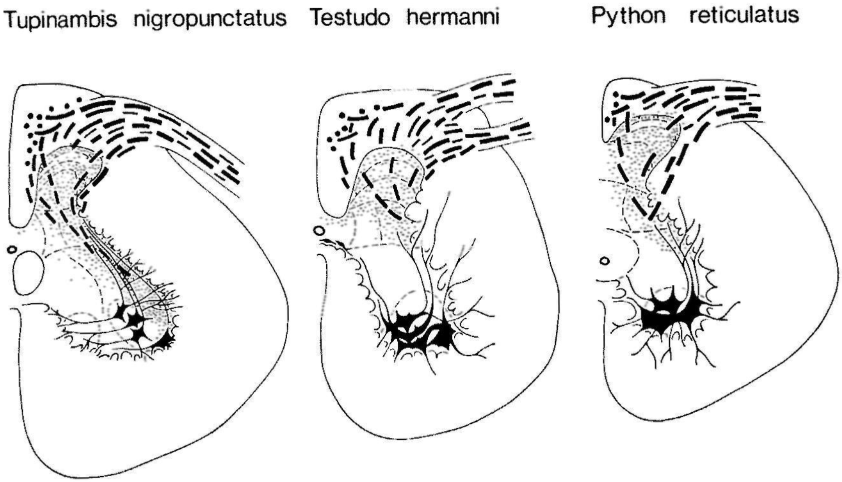


Fig. 23 Schematic diagram summarizing the distribution of the dorsal root fibers within the severed spinal segment in the lizard *Tupinambis nigropunctatus*, the turtle *Testudo hermanni* and the snake *Python reticulatus*. For symbols cf. Fig. 18A. The motoneurons in the ventral horn are also shown.

hermanni and in *Python reticulatus*, almost no fibers were found to extend into the ventral horn.

The present results in the lizard *Tupinambis nigropunctatus* are consistent with data in other lizards presented by Joseph and Whitlock (1968a). These authors studied the dorsal root distribution in various terrestrial vertebrates. In the lizards *Ctenosaura hemilopha* and *Iguana iguana*, primary afferent fibers could be traced along the lateral margin of the ventral horn and in several instances appeared to cascade down along the dorsal dendrites to a point very close to the somata of the motoneurons from which these dendrites originate. A similar dorsal root projection into the ventral horn has been described by Goldby and Robinson (1962, *Lacerta viridis*) and Cruce (1979, *Tupinambis nigropunctatus*). However, in *Caiman sclerops*, almost no fibers were found to extend into the ventral horn (Joseph and Whitlock, 1968a).

The considerable variation in dorsal root distribution has been correlated by Joseph and Whitlock (1968a) with the gross hindlimb structure of the reptiles studied: the caiman limb has no appreciable capacity for fine prehensile movements, whereas the lizards mentioned above possess long multijointed digits which give their limbs a marked prehensile character. Accompanying this adaptive increase Joseph and Whitlock (1968a) claim a closer potential coupling between the primary input and output systems of the spinal cord in lizards than in the caiman and other reptilian orders.

It has already been noted that in reptiles the motoneurons have extensive dendritic trees, which invade the dorsal horn (cf. Banchi, 1903; Joseph and Whitlock, 1968a; Petras, 1976). It seems likely (see also Fig. 23) that in turtles, snakes and caimans the primary afferent fibers establish synaptic contacts with distal parts of the motoneuronal dendritic tree, whereas in lizards more proximal parts are reached. Unfortunately, only few physiological data on afferent-efferent segmental relationships are available in reptiles. The work of Rosenberg (1972, 1974), however, does indicate in the turtle *Testudo graeca* the presence of monosynaptic connections between primary afferents and ventral horn cells, and thus supports the above-mentioned hypothesis.

The primary long ascending fibers in the dorsal funiculus show a gross somatotopical arrangement in such a fashion that fibers of caudal origin are most medial and those joining at more rostral levels are situated more laterally (Kruger and Witkovsky, 1961; Goldby and Robinson, 1962; Ebbesson, 1967, 1969; Joseph and Whitlock, 1968b; Rosenberg, 1974). Following dorsal funicular lesions Ebbesson (1967, 1969) and Pedersen (1973) demonstrated in addition to a projection to the nucleus funiculi dorsalis, a small bundle by-passing this nucleus, and traveling rostrally into the cerebellum. Since no evidence for such a projection was found following dorsal rhizotomies, it seems likely that this pathway consists of non-primary spinal afferents. No evidence for the presence of non-primary afferents to the dorsal funicular nuclei as recently shown in mammals (cf. e.g. Rustioni and Kaufman, 1977), has been found so far in nonmammalian vertebrates.

Comparing the dorsal root projections of various terrestrial vertebrates, one is struck by the tremendous variation (cf. Joseph and Whitlock, 1968a; Ebbesson, 1976a). In frogs, for example, the dorsal root distribution is practically restricted to the dorsal horn. Physiological studies have shown the presence of monosynaptic activation of motoneurons by primary afferents in the frog lumbar cord (cf. e.g. Cruce, 1974; Székely and Czéh, 1976), but not in the thoracic cord (Carlsen and Mendell, 1977). In the pigeon (van den Akker, 1970; Leonard and Cohen, 1975b), the course, extent and pattern of distribution of dorsal root fibers is very similar to that of mammals. In various mammals, as e.g. the opossum (Culberson and Kimmel, 1975), such fibers not only reach the ventral horn, but the contralateral side as well.

It seems likely that, with regard to the contact between primary afferent fibers and spinal motoneurons, in frogs, and in reptiles like turtle, snake and caiman, primary afferent fibers terminate on distal parts of the dendritic trees of motoneurons. In lizards and in pigeons more proximal parts of the motoneuronal dendrites are reached, whereas in mammals also axosomatic synaptic contacts have been found. This further ventral extent of dorsal root fibers is accompanied by a "retraction" of the motoneuronal dendritic trees (cf. Joseph and Whitlock, 1968a; Ebbesson, 1976a). These findings seem to indicate that where dendrites once extended, dorsal root fibers have taken their place (Ebbesson, 1976a).

Finally, it should be noted that in all vertebrates mentioned, primary afferents form only a small percentage of synaptic input to motoneurons (Bodian, 1975; Conradi, 1969; McLaughlin, 1972). The majority of synaptic input to the motor cells is derived from interneurons (cf. e.g. Gelfan et al., 1974).

Introduction

Relatively little is known concerning the propriospinal connections in reptiles. Physiological studies (Shimamura, 1973) in various vertebrates including reptiles (snake, alligator, iguana and turtle) showed that descending propriospinal reflexes from forelimb to hindlimb can be elicited in all reptiles studied, except understandably the snake. In the yellow rat snake *Elaphe obsoleta quadrivittata* a single shock to any dorsal root studied elicited local intersegmental reflexes in a rostral direction for three to four segments, whereas the caudal extension of the reflex was limited to only one or two segments (Shimamura, 1973).

Ascending interlimb reflexes from hindlimb to forelimb have been observed in mammals, birds, turtles, frogs and toads. The turtle *Pseudemys scripta elegans* exhibited a strong crossed forward interlimb reflex. The ascending interlimb reflexes were not altered by spinal transection at the C1-level. These findings imply that the ascending interlimb reflex may involve only propriospinal mechanisms in *Pseudemys*. The neural elements necessary for interlimb coordination have been studied also with physiological techniques in *Pseudemys scripta elegans* by Stein and co-workers (Lennard and Stein, 1977; Stein, 1978) and with the horseradish peroxidase retrograde tracer technique in the lizard *Lacerta galloti* (ten Donkelaar and de Boer - van Huizen, 1978b).

In the present chapter in particular attention will be focussed on the long propriospinal fibers interconnecting the intumescences in the quadrupedal reptiles studied.

Notes on the various anatomical techniques used.

(1) *Anterograde degeneration techniques:*

Anterograde degeneration techniques (Nauta and Gygax, 1954; Fink and Heimer, 1967) are especially useful to study the course and site of termination of ascending propriospinal fibers. The study of the distribution of descending propriospinal fibers is complicated by the fact that hemisections of the spinal cord interrupt not only the descending propriospinal fibers but also the descending supraspinal pathways. In order to eliminate degenerating fibers produced by descending supraspinal systems one has to take advantage of the so-called successive hemisection technique (Sherrington and Laslett, 1903; Giovanelli Barilari and Kuypers, 1969). In *Tupinambis nigropunctatus* and *Testudo hermanni* the descending supraspinal pathways were first interrupted by a high cervical hemisection. After 11 months the debris of the degenerating supraspinal fibers could no longer be demonstrated in the spinal gray matter by means of the silver impregnation techniques. Thus after this period the distribution of the propriospinal fibers descending from the cervical cord could be determined by studying the fiber degeneration in the spinal gray matter resulting from a hemisection just caudal to the cervical intumescence.

Unfortunately, the lizard *Tupinambis nigropunctatus* could not be kept in captivity for the very long survival periods required. However, a comparison of data obtained after high cervical hemisections with those in hemisections just caudal to the cervical intumescence, enabled us to verify the distribution of the descending propriospinal fibers in this lizard.

The distribution of the ascending propriospinal fibers can be determined more directly, i.e. by studying the pattern of ascending fiber degeneration in the spinal gray matter following upper lumbar hemisections.

By placing small lesions in the funiculi the differential distribution of the propriospinal fibers from the different parts of the ventral and lateral funiculi to the spinal gray, especially the motoneuronal area, can be studied: the dense degeneration which occurs in the lateral motoneuronal column following such lesions in e.g. the cat (Sterling and Kuypers, 1968; Rustioni et al., 1971) most probably represents mainly short propriospinal elements, since silver impregnation studies have shown that in the cat, the bulk of the fibers of the descending supraspinal pathways (cf. Nyberg - Hansen, 1966) as well as long propriospinal fibers (Giovanelli Barilari and Kuypers, 1969; McLaughlin, 1972; Matsushita and Ikeda, 1973) avoid the lateral motoneuronal area.

(2) *Retrograde degeneration and tracer techniques:*

The differential location of the cells of origin of fibers in different parts of the ventral and lateral funiculi can be determined by comparing the distribution of chromatolytic cells in the spinal gray of animals in which small lesions have been made in different parts of these funiculi (cf. Sterling and Kuypers, 1968; Molenaar et al., 1974) or by retrograde transport of the enzyme HRP (cf. Molenaar and Kuypers, 1975, 1978; Skinner, 1977).

Typical retrograde cell changes include an obvious dissolution of the Nissl bodies (chromatolysis), swelling of the perikaryon, and a peripheral displacement (eccentricity) of the nucleus. In mammals, these acute retrograde cell changes are most pronounced in young specimens between one and three weeks after axotomy (Brodal, 1940). In the reptiles studied so far, however, a considerably longer survival time appeared to be necessary (Robinson, 1969; ten Donkelaar, 1976a). Retrograde cell changes were observed only in the larger elements; however, the absence of chromatolysis in small cells should be interpreted with reservation because of the extreme difficulty in judging retrograde changes in such neurons. In the material studied, no clear retrograde cell changes were observed, and therefore in the present thesis an attempt to determine the cells of origin of the ascending and descending propriospinal fibers has been made by injecting the enzyme horseradish peroxidase (HRP) unilaterally into various parts of the spinal cord. As regards this retrograde transport technique it should be noted that the labeled cells are clearly visible because of the presence of numerous small brown granules of uniform size which can be demonstrated following incubation in a medium containing hydrogen peroxide and 3,'3-diaminobenzidine tetrahydrochloride. There is little risk of confusing these granules with background staining of the tissue; however, possible sources of confusion are endothelial cells which take up the protein and red blood cells which have endogenous peroxidase activity (LaVail et al., 1973). By light counterstaining with cresylechtviolet these difficulties can be avoided.

A Anterograde degeneration experiments

In order to analyse the course and site of termination of long and short propriospinal fibers, in 8 lizards (*Tupinambis nigropunctatus*), 11 turtles (*Testudo hermanni*) and 12 snakes (*Python reticulatus*) hemisections have been carried out at various levels of the spinal cord (Fig. 24). Some representative experiments in each of the three reptiles mentioned above will now be described. Additional hemicordotomies have been performed in three lizards of the species *Varanus exanthematicus*. It should be noted here that no small lesions were placed in the funiculi to study the differential distribution of the propriospinal fibers from different parts of the various funiculi to the spinal gray.

Results

1) Propriospinal connections in the lizard *Tupinambis nigropunctatus*.

a) *Course and site of termination of descending propriospinal fibers.*

Following a hemisection at the fifth spinal segment (the lesion in addition damaged the medial part of the contralateral ventral funiculus and the greater part of the contralateral dorsal funiculus), caudal to the lesion degenerating fibers were found particularly in the lateral and ventral funiculi (Fig. 25A). Degenerating fibers in the dorsal funiculus were only present in the adjacent segments. Directly caudal to the lesion degenerating fibers appeared throughout the lateral and ventral funiculi. More caudally (e.g. segments 14 and 24, Fig. 25A), however, degenerating fibers were almost only present at the periphery of the lateral and ventral funiculi. These degenerating fibers in the lateral and ventral funiculi could be traced to the most caudal segments

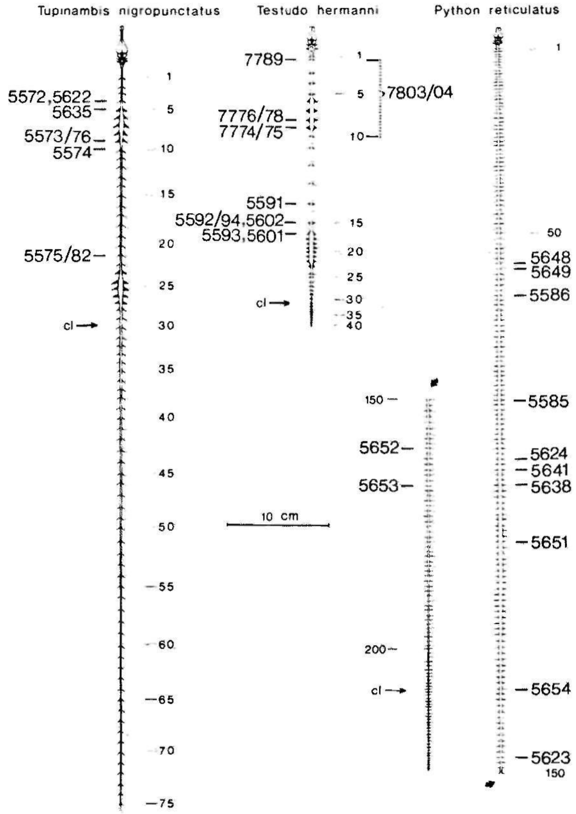
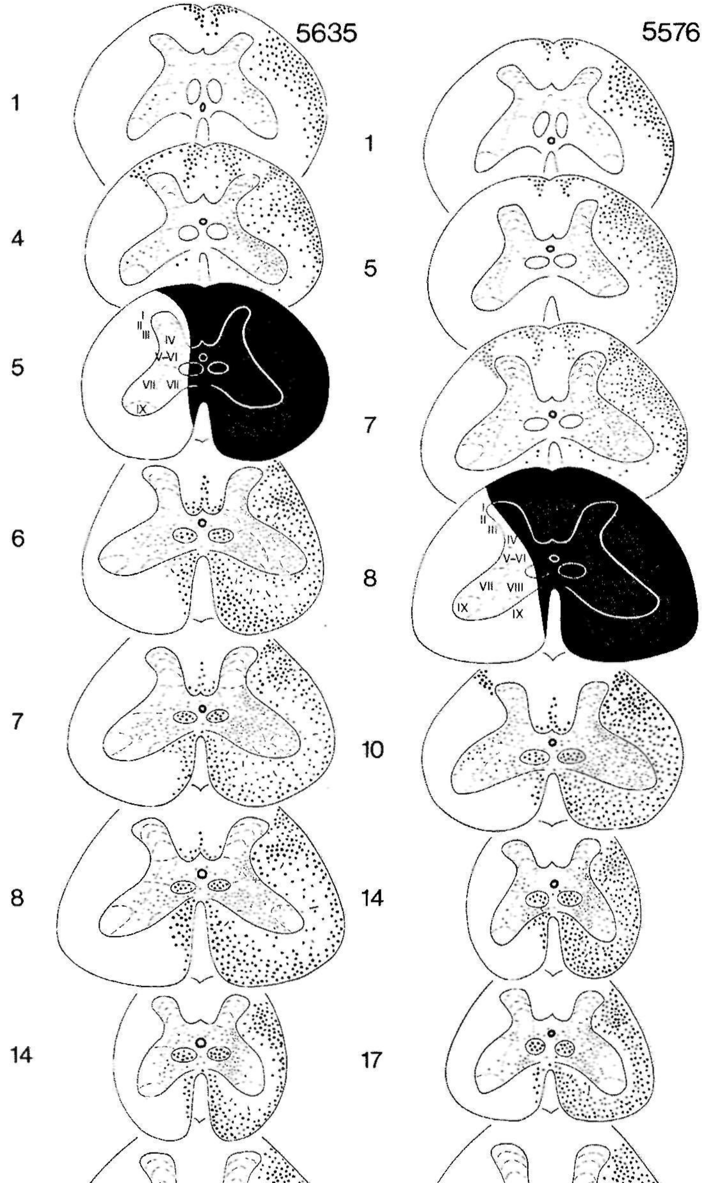


Fig. 24 Schematic representation of the central nervous system in the lizard *Tupinambis nigropunctatus*, the turtle *Testudo hermanni* and the snake *Python reticulatus*, showing the various levels of the hemicordotomies carried out. Abbreviation: cl, level of cloaca.

of the spinal cord. The degenerating fibers in the lateral funiculus occurred at the periphery as a rather diffusely organized system. However, a distinct bundle of degenerating fibers was located in the dorsal part of the lateral funiculus and could be traced as far caudally as the tail segments. This bundle represents the rubrospinal tract. On the basis of their distribution to the spinal gray the degenerating descending fibers could be divided into two groups. The first group, the rubrospinal tract, was found to distribute preferentially to the lateral part of the ipsilateral area V - VI. A second group of fibers situated in the lateral as well as in the ventral funiculus was distributed densely to the ventromedial part of area VII - VIII on both sides. A more or less distinct field of preterminal degeneration was observed in the lateral part of area VII - VIII of about three segments directly caudal to the lesion, particularly ipsilaterally. Some of these endings were also present in the lateral motoneuron column of the above-mentioned spinal segments which constitute the cervical intumescence. No terminal or preterminal degeneration could be traced to the lateral motoneuron column nor to the lateral part of area VII - VIII of the spinal segments involving the lumbar intumescence (e.g. the 24th spinal segment, Fig. 25A).

Lesions in the caudal part of the cervical intumescence (e.g. the eight spinal segment, Fig. 25B) resulted in degenerating descending fibers the distribution of which was roughly the same as those of the higher lesions (e.g. Fig. 25A). Caudal to the lesion degenerating fibers were demonstrated predominantly in the lateral and ventral funiculi, at first throughout these funiculi. More caudally, however, degenerating fibers were particularly present in the superficial zone of the lateral and ventral funiculi. It should be noted, however, that also in the deep, inner zone of these funiculi, degenerating fibers were found. The degenerating descending fibers were distributed bilaterally to the spinal gray. A group of fibers was distributed particularly ipsilaterally to the lateral part of area V - VI, and another group of fibers was distributed bilaterally to the ventromedial part of area VII - VIII. A more or less distinct field of preterminal debris was observed in the lateral part of area VII - VIII in about six segments directly caudal to the lesion. However, a small amount of preterminal degeneration was also present in the lateral motoneuronal cell areas of the lumbar intumescence, particularly contralateral to the lesion (e.g. the 24th spinal segment, Fig. 25B).

Comparing the distribution of degenerating descending fibers after spinal hemisections at segments rostral to the cervical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intumescence with those after spinal hemisections at segments in the caudal part of the cervical intumescence, it was evident that the degenerating descending fibers in the cord of the latter mentioned cases were more diffusely distributed in the lateral and ventral funiculi (Fig. 25B). In addition to the degenerating fibers in the superficial zone of the lateral and ventral funiculi a more deeply located field of degenerating elements was demonstrated in these funiculi. Moreover, in the lumbar intumescence preterminal degeneration was present throughout the ventral horn, some of the preterminal debris could be traced to the lateral motoneuron column, particularly contralaterally.

Short descending propriospinal fibers, which were found directly bordering the gray matter, did not extend further caudally than about six segments. These fibers terminate in the entire ventral horn including the lateral motoneuronal area, predominantly ipsilaterally. Since following hemisections in segments rostral to the cervical intumescence preterminal degeneration was also present in the ventromedial part of area VII - VIII, it was impossible to distinguish between descending propriospinal fibers and descending supraspinal fibers terminating in this part of area VII - VIII. A very limited number of long descending propriospinal fibers, located in the more peripheral portion of the lateral and ventral funiculi, appeared to be distributed contralaterally, but some were also distributed ipsilaterally. These fibers terminate mainly in the ventromedial part of area VII - VIII (contralaterally also more dorsolaterally), but also in the lateral motoneuron cell area. It should be noted, however, that contralateral to the lesion side

Fig. 25 Semidiagrammatic representation of the rostrocaudal distribution of degeneration following a hemisection at the fifth (A) and at the eight (B) spinal segment in the lizard *Tupinambis nigropunctatus*. In this as well as in the figures 26 - 28 and 33 black areas indicate the position and extent of the lesion. Coarse dots and broken lines indicate transversely respectively longitudinally cut degenerating fibers, whereas small dots represent evidence of preterminal degeneration. In addition, the spinal gray areas are indicated by broken lines.

also a limited number of reticulospinal fibers was interrupted. These descending fibers from the reticular formation terminate also in the ventromedial part of area VII - VIII (ten Donkelaar, 1976b; Kusuma et al., 1979).

b) *Course and site of termination of ascending propriospinal fibers.*

In the lizard *Tupinambis nigropunctatus* so far only incomplete hemisections were carried out in the rostral part of the lumbar intumescence. In the lizard in which the hemisection at the 21st spinal segment spared the dorsal funiculus as well as the dorsal parts of the lateral and ventral funiculi, but additionally damaged the ventromedial part of the contralateral ventral funiculus, degenerating fibers were found mainly in the lateral funiculus (Fig. 26A). Directly rostral to the lesion degenerating fibers were present throughout the lateral funiculus as well as in the inner zone of the ventral funiculus (e.g. segment 20, Fig. 26A). More rostrally the bulk of the degenerating ascending fibers in the lateral funiculus was situated superficially. Passing rostrally the density of degenerating fibers in the lateral funiculus decreased considerably; however, a small, remaining contingent of fibers could be traced to the brain stem and diencephalon (cf. Chapter IX). In the spinal cord the majority of the ascending fibers was found to terminate in area VII - VIII. In the segments directly rostral to the lesion and in the thoracic segments studied, the distribution of the degenerating fibers was more laterally located in area VII - VIII than in the cervical enlargement.

In another lizard in which the lesion interrupted the entire dorsal funiculus (Fig. 26B), the degenerating ascending fibers were distributed largely in the same fashion as those in the former case, but they were relatively less dense than in the former.

In cases in which the dorsal funiculus was also damaged (e.g. Fig. 26B), a dense bundle of degenerating fibers was found in the dorsal funiculus throughout the cord rostral to the lesion. In all hemisections in which the dorsal funiculus was entirely or partly interrupted, rostral to the lesion a pattern of degeneration

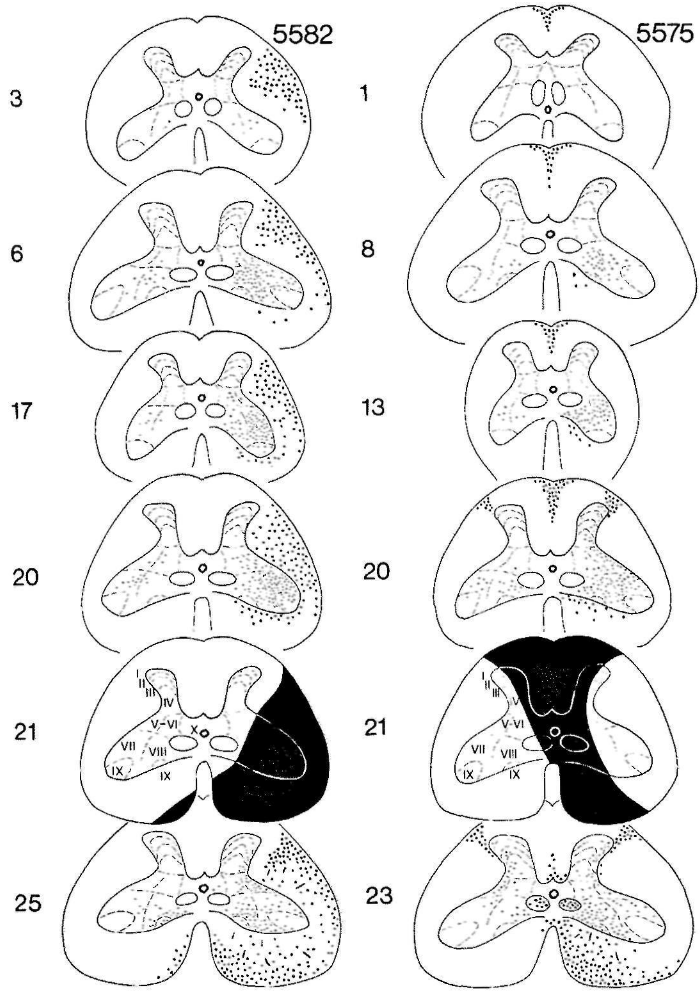


Fig. 26 Semidiagrammatic representation of the rostrocaudal distribution of degeneration following partial hemisections at the 21st (A and B) spinal segment in the lizard *Tupinambis nigropunctatus*. For symbols cf. Fig. 25.

was found in the dorsal funiculus similar to that seen after dorsal root transections (cf. Chapter VII).

Following hemisections at higher levels (e.g. the fifth and the eight spinal segment, Figs. 25A, B), rostral to the lesion the degenerating ascending fibers were distributed largely in the same fashion as those at the lower levels (cf. Fig. 26A, B). Directly rostral to the lesion degenerating fibers were present throughout the lateral funiculus as well as in the inner zone of the ventral funiculus (e.g. the fourth spinal segment, Fig. 25A and the seventh spinal segment, Fig. 25B). More rostrally the bulk of the degenerating ascending fibers in the lateral funiculus was found to occupy a superficial position. Passing rostrally the density of degenerating fibers in the lateral funiculus decreased considerably; however, a small, remaining contingent of fibers could be followed to the brain stem and diencephalon (cf. Chapter IX). Moreover, the dorsal funiculus was also damaged and a dense bundle of degenerating fibers was found in the dorsal funiculus throughout the cord rostral to the lesion. In the spinal cord the majority of the ascending fibers were found to terminate particularly in the dorsolateral part of area VII - VIII, predominantly ipsilaterally.

In summary, short ascending propriospinal fibers, bordering the gray matter, do not extend further rostrally than about six segments. These fibers terminate in the entire ventral horn including the lateral motoneuron column, predominantly ipsilaterally. It seems likely that the preterminal debris of these short ascending propriospinal fibers is located particularly in the dorsolateral part of area VII - VIII. A limited number of long ascending propriospinal fibers, located in the lateral and ventral funiculi more peripherally, appeared to be distributed ipsilaterally. These fibers terminate particularly in the ventromedial part of area VII - VIII, predominantly ipsilaterally.

2) <u>Propriospinal connections in the turtle *Testudo hermanni*.</u>

a) *Course and site of termination of descending propriospinal fibers.*

The combination of data obtained following upper cervical spinal hemisections with those following hemisections directly caudal to the cervical intumescence in the turtle *Testudo hermanni* gives comparable results as regards the differential distribution of descending fibers with those in the lizard *Tupinambis nigropunctatus*. Moreover, successive hemisections were carried out in two turtles (*Testudo hermanni*). The descending supraspinal pathways were first interrupted by a hemisection at the second spinal segment (Fig. 27A). In control experiments after 11 months the debris of the degenerating supraspinal fibers could no longer be demonstrated in the spinal gray and white matter by means of the Nauta-Gygax (1954) and Fink-Heimer (1967) techniques. As a consequence, after this period the distribution of the propriospinal fibers descending from the cervical cord could be determined by studying the fiber degeneration in the cord resulting from a hemisection just caudal to the cervical intumescence (e.g. the 10th spinal segment, Fig. 27A).

Following the second lesion (Fig. 27A) degenerating fibers were found bilaterally in the spinal cord and could be traced to the lumbar enlargement. Directly caudal to the second lesion, degenerating fibers in the lateral funiculus were diffusely arranged. In more caudal segments the density of these degenerating fibers was rapidly declining and they occupied a more centrally situated region in the lateral funiculus. However, a rather distinct bundle of degenerating fibers located at the periphery of the ventral funiculus could be traced to the caudal segments of the cord. These fibers, which were spared by the first lesion and damaged by the second lesion, represent the reticulospinal tract and partly the vestibulospinal tract. Degenerating descending fibers in the dorsal funiculus could only be traced over four segments caudal to the second lesion.

In the thoracic segments studied the bulk of degenerating fibers was present on the side of the hemisection. The majority

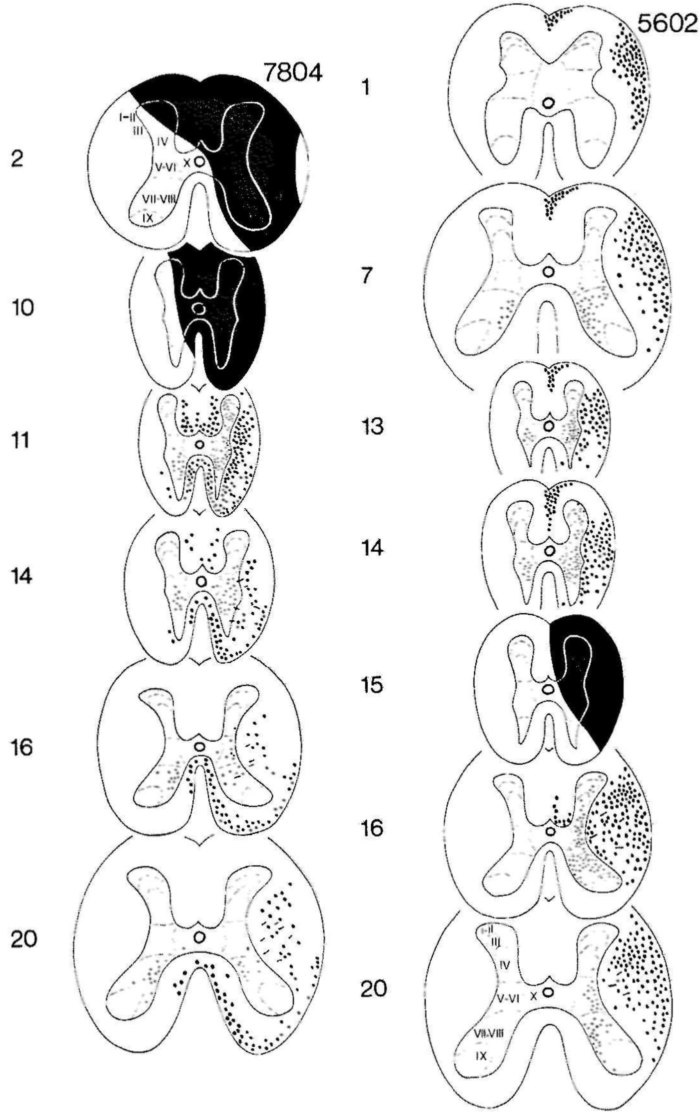


Fig. 27 Semidiagrammatic representation of the distribution of the degenerating descending and ascending fibers following hemisections in the turtle *Testudo hermanni*. A, after a successive hemisection (the first lesion at the second spinal segment and the second lesion at the 10th spinal segment). B, following a hemisection at the 15th spinal segment. For symbols see Fig. 25.

of these degenerating descending fibers were found to terminate in areas V - VI and VII - VIII which constitute the small intermediate zone of the thoracic cord. Contralaterally, a more or less distinct concentration of preterminal debris was present in the ventromedial part of the intermediate zone. Preterminal degeneration was also observed in the spinal gray of the lumbar intumescence, particularly bilaterally in the medial part of area VII - VIII (e.g. segment 20, Fig. 27A). In part this preterminal degeneration is probably due to the involvement of reticulospinal as well as of vestibulospinal fibers.

In summary, short descending propriospinal fibers were found directly bordering the spinal gray. These fibers did not extend further caudally than about four segments and were found to terminate in the entire ventral horn, predominantly ipsilaterally. A very limited number of long descending propriospinal fibers located more peripherally in the lateral and ventral funiculus appeared to be distributed to the ventromedial part of area VII - VIII. It should be noted, however, that the exact site of termination of these long descending propriospinal fibers could not be demonstrated due to the involvement of descending pathways from the brain stem which also terminate in the ventromedial part of area VII - VIII (ten Donkelaar, 1976b).

b) *Course and site of termination of ascending propriospinal fibers.*

The distribution of long ascending propriospinal fibers in the turtle *Testudo hermanni* could be demonstrated by studying the ensuing degeneration in the spinal cord following hemisections just rostral to the lumbar intumescence (e.g. the 15th spinal segment, Fig. 27B). This hemisection was restricted to one side of the cord, however, the lesion largely spared the ventral funiculus. The bulk of degenerating ascending fibers was present on the side of the hemisection. Just rostral to the lesion degenerating fibers in the lateral funiculus were diffusely organized. The density of these degenerating ascending fibers decreased considerably in more rostral segments, particularly in the regions bordering the gray matter. Peripherally, degenerating ascending fibers in the lateral funiculus could be traced as far as the brain stem and diencephalon (see Chapter IX). Degenerating fibers adjacent to the gray matter could only be followed into the thoracic cord, at least as far as three segments rostral to the lesion. Degenerating fibers in the dorsal funiculus were found throughout the cord rostral to the lesion, similar to the pattern of degeneration seen after dorsal root transections (cf. Chapter VII).

In the spinal cord the majority of the preterminal debris was observed in the intermediate zone of the thoracic segments (areas V to VIII), and in the medial part of area VII - VIII in the cervical enlargement (e.g. the seventh spinal segment, Fig. 27B). No terminal debris could be observed in the spinal gray of the upper cervical segments.

3) Propriospinal connections in the snake *Python reticulatus*.

In the snake *Python reticulatus* hemisections were carried out at various levels of the spinal cord (Fig. 24). A representative experiment, viz., an almost complete hemicordotomy at the 57th spinal segment is illustrated in figure 28. The lesion involved also the most medial part of the contralateral ventral funiculus.

Caudal to this hemicordotomy degenerating fibers were present in the lateral and ventral funiculi, contralaterally only in the most medial part of the ventral funiculus. In segments directly caudal to the lesion degenerating fibers were found throughout the lateral and ventral funiculi. Passing further caudalwards degenerating fibers were present only in the superficial zone of the lateral funiculus and in the superficial two-thirds of the ventral funiculus. In the dorsal funiculus degenerating fibers could only be followed for one segment caudal to the lesion. Directly caudal to the lesion degenerating elements were found distributed predominantly to the ipsilateral area VII - VIII. Only a small amount of preterminal debris was observed in more dorsal areas. Passing further caudalwards preterminal and terminal degeneration was only present in the ventromedial part of area VII - VIII.

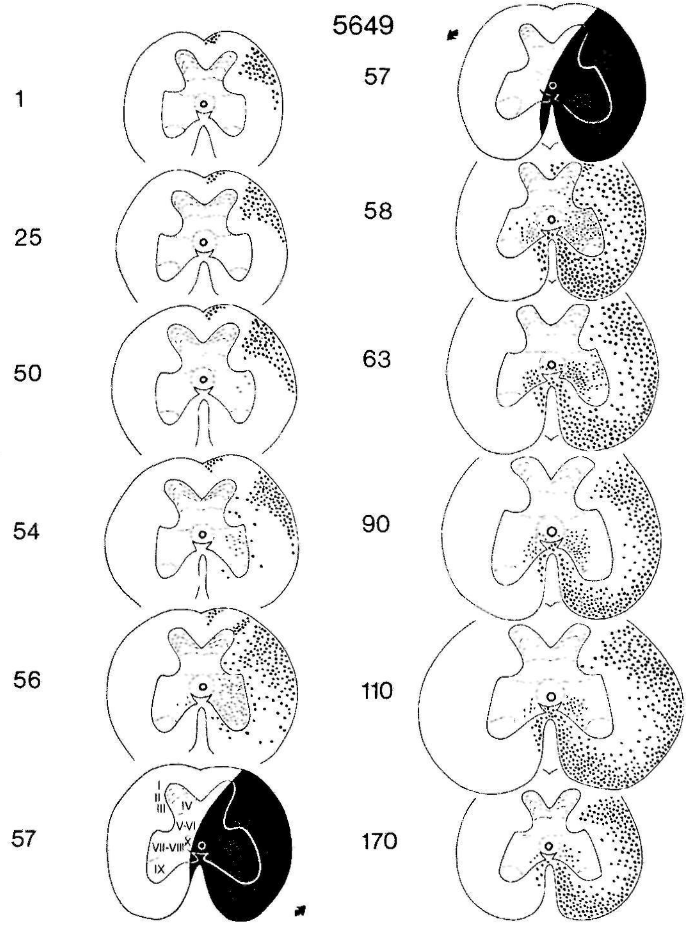


Fig. 28 Semidiagrammatic representation of the rostrocaudal distribution of degeneration following a hemisection at the 57th spinal segment in the snake *Python reticulatus*. For symbols cf. Fig. 25.

Following high hemicordotomies in *Python reticulatus* (see ten Donkelaar, 1976b, Fig. 2) degenerating fibers were present in the superficial zone of the lateral funiculus and in the superficial two-thirds of the ventral funiculus which terminate in the ventromedial part of area VII - VIII. Therefore, it can be concluded from the present experiments that the inner zone of the lateral and ventral funiculi caudal to the lesion is occupied by short descending propriospinal fibers. The degenerating fibers present in about six segments caudal to the lesion terminate throughout the ventral horn including area IX. It should be noted, however, that the presence of long descending propriospinal fibers cannot be demonstrated with the technique employed.

Rostral to the lesion diffusely distributed degenerating fibers were found in the lateral funiculus. The inner zone of the ventral funiculus and the dorsal funiculus also contained a few degenerating fibers. The density of these degenerating ascending fibers decreased considerably in more rostral segments, particularly in the regions bordering the gray matter. Degenerating fibers in the deepest part of the white matter adjacent to the spinal gray, extended over four segments rostral to the lesion. Directly rostral to the lesion the degenerating ascending fibers were distributed to the entire ipsilateral area VII - VIII, particularly dorsolaterally. Preterminal and terminal degeneration could not be traced beyond spinal segment 50.

In summary, so far only short propriospinal fibers have been observed in the snake *Python reticulatus* following spinal cord hemisections. These fibers are directly bordering the gray matter and they extend rostrally as well as caudally for about six segments. These short propriospinal fibers probably terminate particularly in the dorsolateral part of the ipsilateral area VII-VIII.

B Experiments based on retrograde axonal transport of the enzyme horseradish peroxidase (HRP)

In 9 lizards (2 *Tupinambis nigropunctatus* and 7 *Varanus exanthematicus*), 14 turtles (6 *Testudo hermanni* and 8 *Pseudemys* *scripta elegans*) and 5 snakes (*Python reticulatus*), 3 - 8 unilateral injections were made of 0,1 ul HRP (a 20% solution) into various levels of the spinal cord in order to analyse the location of cells of origin of propriospinal (this chapter) as well as supraspinal (cf. Chapter IX) fibers. Some representative experiments in each of the three reptiles mentioned above will now be described. Additionally, in two turtles (*Pelomedusa subrufa*) a single injection of 0,2 ul HRP was made into the gray matter of the lumbar intumescence.

Results

1) Cells of origin of propriospinal fibers in the lizards *Tupinambis nigropunctatus* and *Varanus exanthematicus*.

HRP-injections into the 23rd spinal segment of *Tupinambis nigropunctatus* (Fig. 29), i.e. the rostral part of the lumbar intumescence, resulted in the presence of retrogradely labeled neurons caudal as well as rostral to the injected segment. In this case the needle penetrations surrounded by dense accumulations of HRP-reaction products involved the lateral funiculus as well as the lateral part of the ventral funiculus, both only ipsilaterally. Caudal to the injected segment labeled neurons were present particularly in the adjacent spinal segments. In segment 24 retrogradely labeled cells were found throughout the spinal gray, particularly ipsilaterally. More caudally (segments 25 - 27, Fig. 29) a rather massive accumulation of labeled neurons was found in area VII - VIII, especially contralaterally. Ipsilateral to the injection side labeled cells in area VII - VIII were present predominantly ventromedially, whereas contralaterally retrogradely labeled neurons in this area were situated more laterally. Caudal to the injected segment labeled neurons were located also bilaterally in the medial part of area V - VI as well as in various areas of the dorsal horn. Passing caudally the number of labeled neurons in the dorsal horn was rapidly declining.

Rostral to the injected segment retrogradely labeled neurons were found as far cranial as the fourth spinal segment, particularly

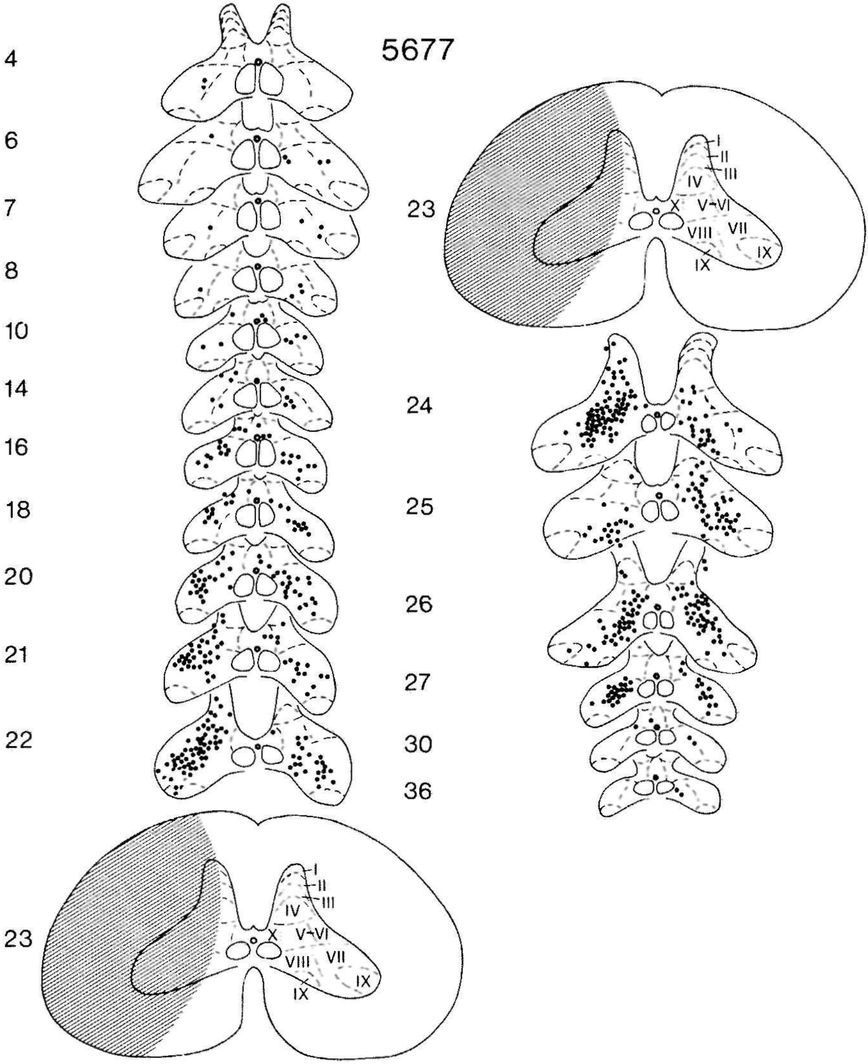


Fig. 29 Distribution of retrogradely labeled neurons throughout the spinal cord of the lizard *Tupinambis nigropunctatus* after HRP-injections into the 23rd spinal segment. Each level represents the composite of plots of 10 consecutive sections. In this as well as in the figures 30 - 32 and 36 - 37, the shaded areas indicate the extent of the injections. The spinal gray areas are indicated by broken lines.

ipsilaterally. In the spinal segments immediately rostral to the HRP-injections (e.g. segment 22, Fig. 29) labeled neurons were situated bilaterally in area VII - VIII and in the medial part of area V - VI, particularly ipsilaterally. In the ipsilateral dorsal horn labeled neurons were found only in the adjacent spinal segments (segments 20 - 22, Fig. 29). Contralaterally, only a few labeled cells were observed in the dorsal horn. Passing rostrally the number of labeled neurons in area V - VI was rapidly declining, however, in area VII - VIII labeled neurons were found as far rostrally as the fourth spinal segment. In the cervical enlargement (see segments 6 - 8, Fig. 29) retrogradely labeled neurons were found particularly contralaterally in the medial part of area VII - VIII.

In conclusion, the cells of origin of long and short descending propriospinal fibers to the lumbar intumescence have been demonstrated in the lizard *Tupinambis nigropunctatus*. The cells of origin of pathways descending from supraspinal levels will be described in Chapter IX. In the lizard *Varanus exanthematicus* comparable results were obtained. The distribution of retrogradely labeled cells after lumbar cord injections strongly suggests the presence of long propriospinal fibers from the cervical to the lumbar intumescence. The labeled neurons caudal to the injected segment comprise cells of origin of short and long ascending propriospinal fibers as well as cells of origin of long ascending pathways to the brain stem. It should be noted here that in the lizard *Lacerta galloti* the cells of origin of the latter pathways to the brain stem are predominantly situated in the contralateral areas V - VI and VII - VIII (ten Donkelaar and de Boer - van Huizen, 1978b). Therefore, the labeled cells found ipsilaterally as well as in other areas contralaterally, are probably cells of origin of short propriospinal fibers. The cells of origin of long ascending propriospinal fibers are difficult to demonstrate with the present technique. It should be noted, however, that following unilateral HRP-injections into the spinal gray of the ninth spinal segment in the lizard *Varanus exanthematicus* a few labeled neurons were found in the lumbar intumescence in the contralateral area VII - VIII (ten Donkelaar, personal communication).

2) Cells of origin of propriospinal fibers in the turtles *Testudo hermanni* and *Pseudemys scripta elegans*.

Following HRP-injections into the 16th spinal segment of *Testudo hermanni* (Fig. 30) labeled neurons were present particularly in the adjacent spinal segments. In more caudal and rostral segments the number of labeled cells was rapidly declining. In this case the needle tracks surrounded by dense accumulations of HRP-reaction products were present mainly unilaterally, but in addition involved the most medial part of the contralateral dorsal funiculus. In the lumbar intumescence (e.g. segments 18 and 20, Fig. 30) labeled cells were found bilaterally. Ipsilaterally, retrogradely labeled neurons were located predominantly in area V - VI. Contralateral to the injection side, retrogradely labeled neurons were found especially in the medial part of area VII - VIII. Labeled cells were also observed in area V - VI and area VII - VIII, whereas only a few labeled cells were found in areas I to IV, IX and X.

Rostral to the injected segment labeled neurons were present bilaterally and could be demonstrated even in the first spinal segment. In the thoracic segments studied (e.g. segments 10 and 14, Fig. 30) retrogradely labeled neurons were found in areas V to VIII, however, contralaterally, labeled cells were also present in area IV. In the cervical intumescence (e.g. segments 6 and 8, Fig. 30) a few labeled neurons were present bilaterally. Contralaterally, the retrogradely labeled neurons were found in the medial part of area VII - VIII. Ipsilaterally they were located more laterally in area VII - VIII.

In conclusion, the present experiments have demonstrated in the turtle *Testudo hermanni* the cells of origin of short and long descending propriospinal fibers to the lumbar intumescence. The labeled neurons caudal to the injections include cells of origin of short and long ascending propriospinal fibers as well as cells

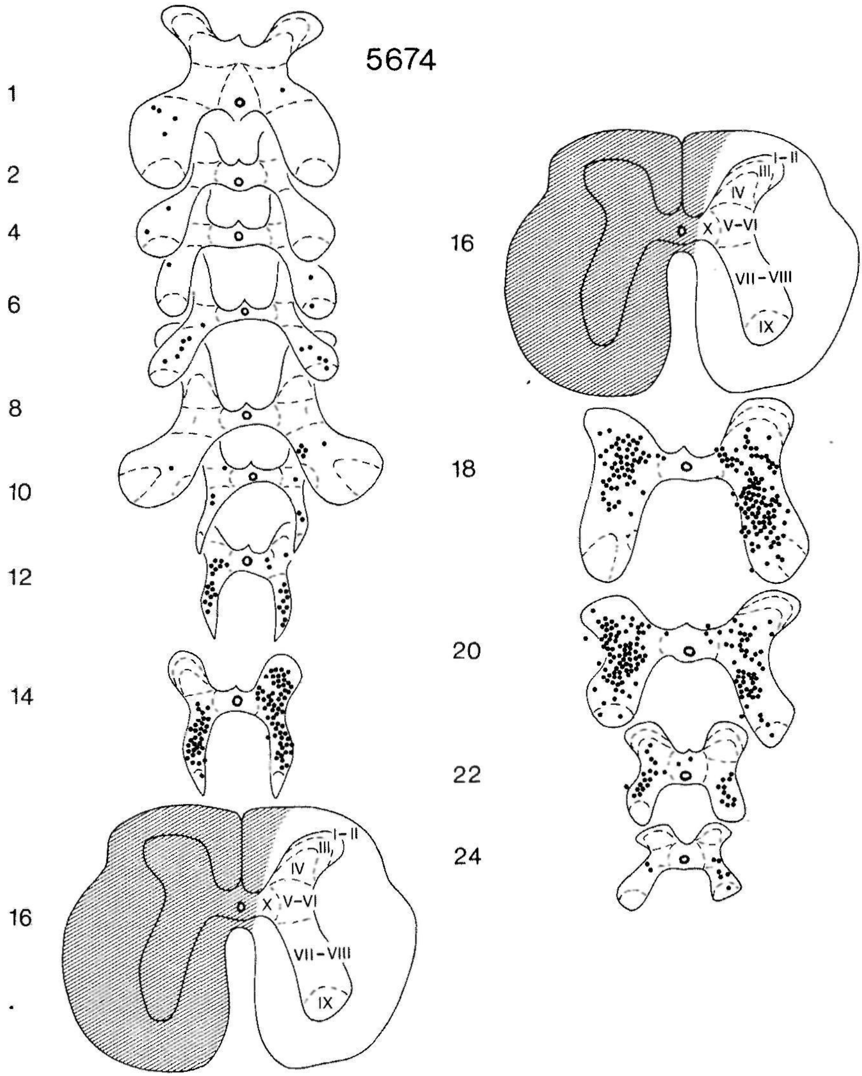


Fig. 30 Distribution of retrogradely labeled neurons throughout the spinal cord of the turtle *Testudo hermanni* after HRP-injections into the 16th spinal segment. Each level represents the composite of plots of 10 consecutive sections. For symbols cf. Fig. 29.

of origin of ascending pathways to the brain stem. In the turtle *Pseudemys scripta elegans* comparable results were obtained. Moreover, following HRP-injections in the latter turtle into the spinal gray and ventral funiculus of the ninth segment (the lateral funiculus was not involved), a few labeled neurons were found in the lumbar intumescence, particularly contralaterally, in area VII - VIII. Since the long ascending fibers from the spinal cord to the brain stem pass by way of the lateral funiculus, it seems likely that these elements represent cells of origin of long ascending propriospinal fibers from the lumbar intumescence to the cervical enlargement.

3) Cells of origin of propriospinal fibers in the snake *Python reticulatus*.

HRP-injections into the 73rd spinal segment of *Python reticulatus* (Fig. 31) resulted in the presence of retrogradely labeled neurons caudal as well as rostral to the injected segment. In this case the needle tracks surrounded by dense accumulations of HRP-reaction products were present mainly unilaterally, but in addition involved part of the contralateral dorsal funiculus. Labeled neurons were present particularly in the adjacent spinal segments. Caudal to the injections retrogradely labeled neurons were mainly located in area VII - VIII bilaterally. These labeled cells caudal to the injected segment probably represent cells of origin of short ascending propriospinal fibers as well as tract cells, i.e. the cells of origin of long spinal fibers ascending to the brain stem and diencephalon. In the material studied (five cases) labeled cells could not be demonstrated beyond 11 segments caudal to the injected segment.

In the experiment illustrated in Figure 31, rostral to the injected segment retrogradely labeled neurons were present particularly ipsilaterally in area VII - VIII. Further rostrally the number of retrogradely labeled cells was rapidly declining and in the series of five cases studied labeled cells could not be demonstrated beyond 10 segments rostral to the injected segment.

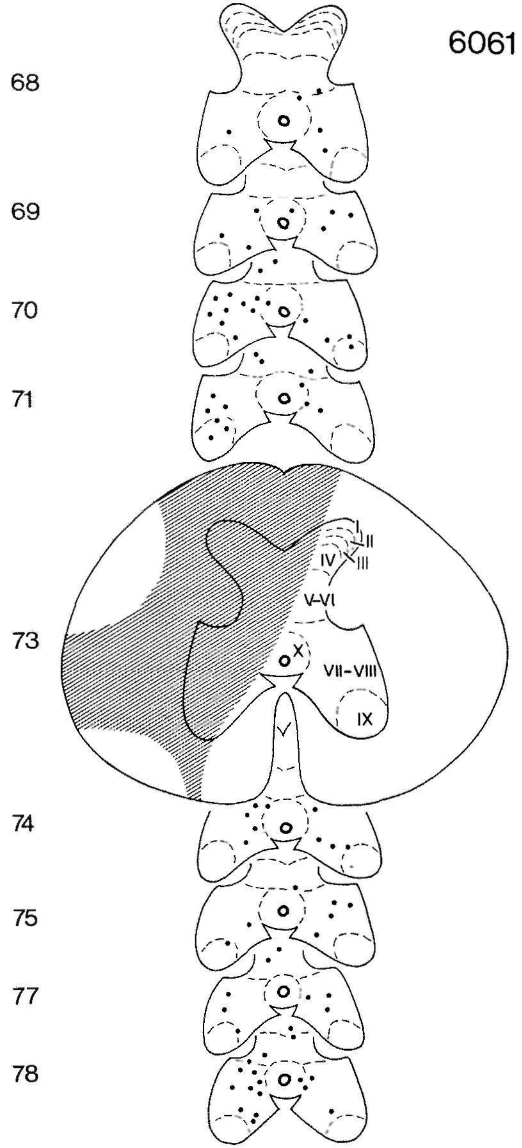


Fig. 31 Distribution of retrogradely labeled neurons throughout the spinal cord of the snake *Python reticulatus* after HRP-injections into the 73rd spinal segment. Each level represents the composite of plots of 10 consecutive sections. For symbols see Fig. 29.

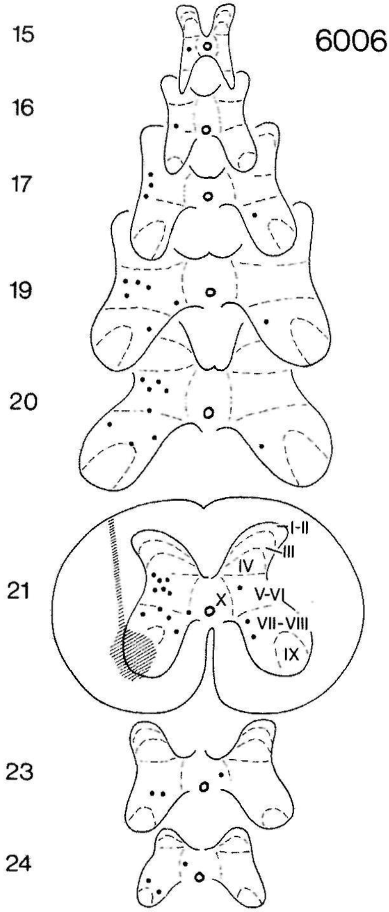


Fig. 32 Semidiagrammatic representation of the distribution of the labeled neurons in the spinal cord after small HRP-injections into the lateral motoneuronal cell group at the 21st spinal segment of the turtle *Pelomedusa subrufa*. Each level represents the composite of plots of 3 consecutive sections. For symbols cf. Fig. 29.

4) Cells of origin of fibers projecting to the lumbar motoneuronal cell group in the turtle *Pelomedusa subrufa*.

The findings in the preceding HRP-experiments (Chapter VIIIB) indicate that neurons in area V - VI and in area VII - VIII give rise especially to short propriospinal fibers and propriospinal fibers interconnecting the intumescences, respectively. In view of previously described anterograde degeneration findings (Chapter VIIIA) these fibers are distributed to both the intermediate zone and the motoneuronal cell groups. However, the HRP-injection technique as used in the previous series of experiments did not allow for a distinction between the cells of origin of propriospinal fibers projecting to the motoneuronal cell groups from those of fibers projecting to the intermediate zone. Therefore, in an additional group of experiments small HRP-injections were made into the motoneuronal cell group of the lumbar intumescence of the turtle *Pelomedusa subrufa* (segment 21, Fig. 32).

The HRP-injection was mainly restricted to the motoneuronal cell group, but also involved the adjoining part of the ventral and lateral funiculi. The distribution of the retrogradely labeled neurons was largely restricted to the lumbar cord (segments 15 - 24, Fig. 32), where they were situated almost exclusively in the intermediate zone. The bulk of the labeled neurons occurred ipsilaterally in areas V to VIII, while some labeled neurons were present contralaterally in area VII - VIII, and very few in area X.

These findings suggest that the fibers which are distributed to the lumbar motoneuronal cell group, at least of the 21st spinal segment, are derived especially from neurons in the intermediate zone of lumbar spinal cord.

Discussion

In the present chapter an attempt has been made to analyse the propriospinal connections in reptiles using different types of locomotion, viz., the lizard *Tupinambis nigropunctatus*, the turtle *Testudo hermanni* and the snake *Python reticulatus*. More in particular attention has been focussed on the long propriospinal fibers interconnecting the enlargements of the spinal cord in the quadrupedal reptiles studied. Anterograde degeneration techniques have been used to study the course and site of termination of ascending and descending propriospinal fibers, whereas the cells of origin of these pathways have been determined with the HRP-technique.

Comparison of the distribution pattern of degenerating fibers after lesions directly caudal to the cervical intumescence in *Tupinambis nigropunctatus* and *Testudo hermanni* with those following upper cervical hemisections, does indicate the presence of short as well as long descending propriospinal fibers. Successive hemisections carried out in *Testudo hermanni* confirm this projection. Short descending propriospinal fibers were found directly bordering the spinal gray. These fibers did not extend further caudally than about four to six segments and were found to terminate in the entire ventral horn, predominantly ipsilaterally. A limited number of long propriospinal fibers descending from the cervical intumescence located more peripherally in the lateral and ventral funiculi appeared to be distributed to the ventromedial part of area VII - VIII in the lumbar intumescence. Moreover, in *Tupinambis nigropunctatus* a small amount of preterminal degeneration was found in the lateral motoneuron column of the lumbar intumescence, particularly contralaterally.

The course and site of termination of ascending degenerating fibers following lumbar hemisections in the lizard *Tupinambis nigropunctatus* and in the turtle *Testudo hermanni*, have been demonstrated. Propriospinal as well as long spinal fibers ascending to the brain stem and diencephalon have been shown. Short propriospinal fibers ascending to segments just rostral to the lesion are directly bordering the gray substance, whereas fibers ascending to more rostral segments are somewhat more peripherally situated. Long propriospinal fibers are still more peripherally located and even reach the cervical intumescence, passing via the ventral as well as via the lateral funiculus. The short propriospinal fibers terminate in the entire ventral horn including the lateral motoneuronal area, predominantly ipsilaterally. Long ascending propriospinal fibers terminate particularly in the ventromedial part of area VII - VIII.

The cells of origin of short as well as long descending propriospinal fibers to the lumbar enlargement have been demonstrated by injecting HRP into the lumbar intumescence of lizards and turtles. Labeled neurons in the thoracic segments were present particularly in the lateral part of area VII - VIII, predominantly ipsilaterally. Labeled neurons in the cervical intumescence were present in the medial part of area VII - VIII, particularly contralateral to the injection side.

The cells of origin of ascending propriospinal pathways are difficult to demonstrate with the present technique, since the HRP-injections also damaged axons of tract cells, i.e. cells of origin of long spinal fibers to the brain stem and diencephalon. In a recent study in the lizard *Lacerta galloti* (ten Donkelaar and de Boer - van Huizen, 1978b) it has been shown that the cells of origin of these ascending supraspinal pathways are predominantly situated in the contralateral areas V - VI and VII - VIII. Therefore, it seems likely that the large remaining part of labeled neurons caudal to the injected segment represent cells of origin of short and long propriospinal fibers. By injecting small amounts of HRP into the spinal gray of the cervical intumescence of the lizard *Varanus exanthematicus* and the turtle *Pseudemys scripta elegans* the cells of origin of long propriospinal fibers in the lumbar intumescence projecting to the cervical enlargement have been demonstrated in part. These cells were found in the ventromedial part of area VII - VIII, particularly contralaterally.

In the snake *Python reticulatus* so far only short propriospinal fibers have been observed following spinal hemisections and HRP-injections into the cord, confirming the results of Shimamura (1973) for the yellow rat snake.

In conclusion, in the quadrupedal reptiles studied short as well as long propriospinal fibers have been demonstrated. In the snake *Python reticulatus* which lacks enlargements only short propriospinal fibers have been found. The long propriospinal fibers in lizards and turtles terminate in the ventromedial part of area VII - VIII, i.e. the same site of termination of the medial system of descending pathways from the brain stem (ten Donkelaar, 1976b). Such a coincidence of the termination area of the long propriospinal fibers with that of descending brain stem pathways has also been observed in the cat (Giovanelli Barilari and Kuypers, 1969).

It seems likely that the organization of the propriospinal connections in reptiles is readily comparable to that in mammals. Findings in the cat suggest that the propriospinal connections follow the same organizational principles as those governing the descending brain stem pathways (Kuypers, 1973). The bulk of the propriospinal fibers is short and terminates in nearby segments. However, the fibers in the ventral funiculus which are distributed to the ventromedial portion of the intermediate zone (lamina VIII) travel over much longer distances, some of them interconnecting the enlargements. These long propriospinal fibers are mainly derived from neurons situated in lamina VIII (Molenaar and Kuypers, 1978). It seems probable that the propriospinal neurons connected primarily to the lateral descending systems as well as the proprio-spinal neurons connected to the medial ones form relatively specialized pathways for the transmission of signals to the moto-neurons (Kuypers, 1973; Kostyuk, 1975, 1976): the former system projects predominantly to motoneurons innervating distal extremity muscles and the intrinsic extremity flexors, whereas propriospinal neurons connected to the medial descending system project especially to motoneurons innervating axial and proximal limb muscles.

The demonstration of long propriospinal connections in lizards and turtles, i.e. quadrupedal reptiles which move their limbs in a particular diagonal pattern (R.C. Snyder, 1952; Bellairs, 1970; Guibé, 1970), suggests that these pathways represent intrinsic links between motor centers controlling hindlimb and forelimb movements. The presence of such motor centers controlling the movements of a given limb has been proposed in mammals (Graham Brown, 1911, 1914; Miller and van der Burg, 1973; Halbertsma et al., 1976; Wetzel and Stuart, 1976; Miller and Scott, 1977) as well as in the turtle *Pseudemys scripta elegans* (Stein, 1976, 1978; Lennard and Stein, 1977). Experimental data in this turtle are consistent with the hypothesis that (1) each limb has its neural control center resident mainly in the spinal cord, and (2) the neural elements necessary for interlimb coordination are not dependent upon supraspinal connections. It seems likely that for quadrupedal reptiles also the following working hypothesis can be applied which has been developed in mammals. It is suggested that the long ascending propriospinal pathways contribute facilitation to the flexors of the forelimb, once the ipsilateral hindlimb has begun the first extension leading to its placing on the ground. The influence of long descending propriospinal fibers is less clear (cf. Miller and van der Burg, 1973; Jankowska et al., 1974).

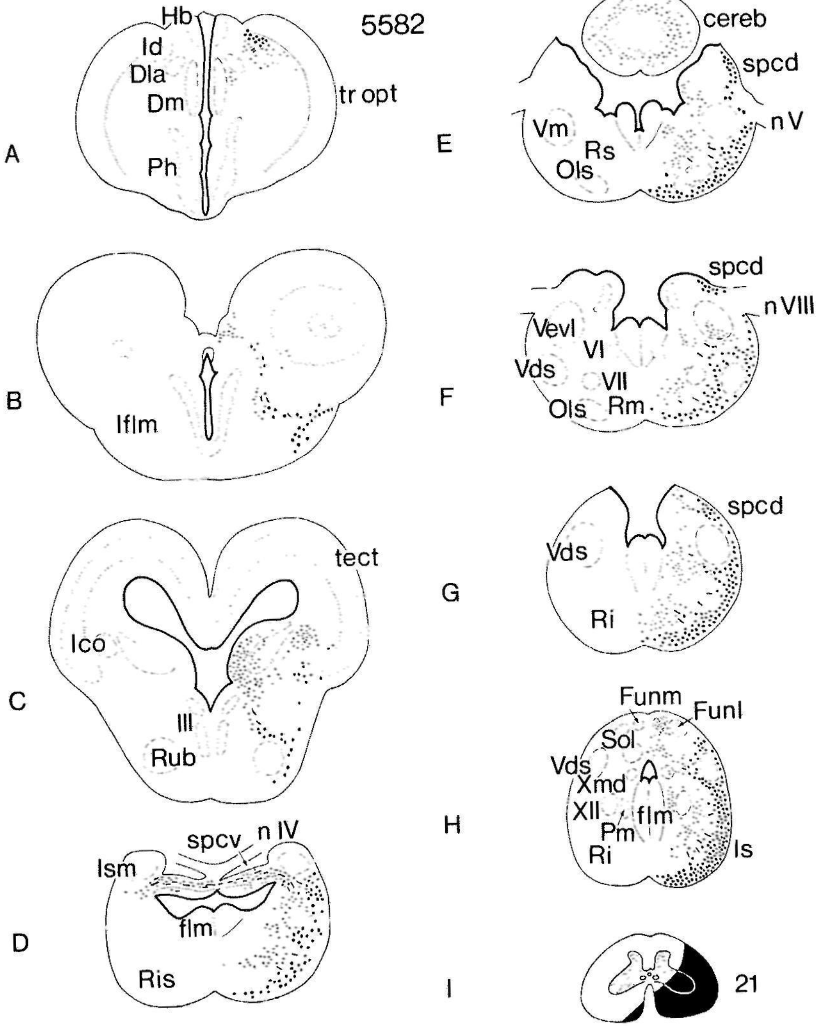
It seems likely that the several locomotor patterns seen in snakes (cf. Gans, 1966; Bellairs, 1970; Guibé, 1970) involve short propriospinal fibers, since longer intraspinal pathways could not be demonstrated.

A Ascending spinal projections to the brain stem and diencephalon

The course and site of termination of ascending spinal fibers to the brain stem and diencephalon have been extensively studied in various reptiles by means of silver impregnation techniques (cf. e.g. Ebbesson, 1967, 1969). As regards the course and site of termination of these long ascending fibers the present findings in the three reptiles studied are remarkably similar considering the diverse modes of progression of the species. Because of similarity in projection pattern of these long ascending fibers, the following brief description (Fig. 33) for the lizard *Tupinambis nigropunctatus* is presented as an example.

Following a lumbar (21st spinal segment) hemicordotomy, degenerating fibers could be observed as far rostral as the diencephalon. Immediately rostral to the lesion degenerating fibers

Fig. 33 Semidiagrammatic representation showing the course and site of termination of the ascending degenerating fibers in the brain stem and diencephalon following a hemisection at the 21st spinal segment in the lizard *Tupinambis nigropunctatus*. For symbols cf. Fig. 25. Abbreviations: cereb, cerebellum; Dla, nucleus dorsolateralis thalami; Dm, nucleus dorsomedialis thalami; flm, fasciculus longitudinalis medialis; Funl, nucleus funiculi dorsalis pars lateralis; Funm, nucleus funiculi dorsalis pars medialis; Hb, habenula; Ico, nucleus intercollicularis; Id, nucleus intermedius dorsalis thalami; Iflm, nucleus interstitialis of the flm; Ism, nucleus isthmi pars magnocellularis; ls, lemniscus spinalis; n IV, nervus trochlearis; n V, nervus trigeminus; n VIII, nervus octavus; Ols, oliva superior; Ph, nucleus periventricularis hypothalami; Pm, nucleus parvocellularis medialis; Ri, nucleus reticularis inferior; Ris, nucleus reticularis isthmi; Rm, nucleus reticularis medius; Rs, nucleus reticularis superior; Rub, nucleus ruber; Sol, nucleus tractus solitarii; spcd, tractus spinocerebellaris dorsalis; spcv, tractus spinocerebellaris ventralis; tect, tectum mesencephali; tr opt, tractus opticus; Vevl, nucleus vestibularis ventrolateralis; III, nucleus nervi oculomotorii; Vds, nucleus descendens nervi trigemini; Vm, nucleus motorius nervi trigemini; VI, nucleus nervi abducentis; VII, nucleus (motorius) nervi facialis; Xmd, nucleus motorius dorsalis nervi vagi; XII, nucleus nervi hypoglossi.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were found in all funiculi. The bulk of the degenerating ascending fibers to the brain stem was situated in the lateral funiculus. No degenerating fibers reach the brain stem by way of the ventral funiculus. The degenerating fibers in the dorsal funiculus were found to terminate in the nucleus funiculi dorsalis pars medialis. The degenerating fibers to the brain stem and diencephalon situated in the lateral funiculus have been designated by Ebbesson (1967) as the lemniscus spinalis. The lateral funiculus contains in addition spinocerebellar fibers. It should be noted here that in the spinal cord and in the caudal part of the brain stem the spinal lemniscus and the spinocerebellar tracts are indistinguishable following hemicordotomies.

Two spinocerebellar tracts, a dorsal and a ventral one, were found in the present experiments, both ascending via the lateral funiculus. In the rhombencephalon (Fig. 33G) the dorsal spino-cerebellar tract separated off from the spinal lemniscus and could be traced to the cerebellum. The ventral spinocerebellar tract (Fig. 33D) extends more rostrally than the dorsal one, and partly decussates in the velum medullare anterius.

The spinal lemniscus can be divided into a) spinorhombencephalic, b) spinomesencephalic and c) spinothalamic projections. The spino-rhombencephalic projection is mainly composed of spinoreticular fibers. Many of the fibers of the spinal lemniscus appeared to pass medially and preterminal degeneration was found in the ipsi-lateral reticular formation. Most ofthese spinoreticular fibers were found to discharge into the nucleus reticularis inferior (Figs. 33G, H) and the caudal part of the nucleus reticularis medius (Fig. 33F). A much less dense projection of preterminal degeneration was demonstrated to the rostral part of the latter nucleus, to the nucleus reticularis superior (Fig. 33E) and to the nucleus reticularis isthmi (Fig. 33D). Only a few fibers were found to terminate in the contralateral reticular formation. In the brain stem in addition spinal projections were demonstrated to the nucleus parvocellularis medialis, to the nucleus motorius dorsalis nervi vagi, to the nucleus tractus solitarii, to the ventrolateral vestibular nucleus and around the nucleus motorius nervi facialis. Preterminal degeneration at mesencephalic levels was found particularly in the intercollicular nucleus and in the periventricular gray (Fig. 33C). The most rostral focus of preterminal degeneration observed was found in the dorsal thalamus (Fig. 33A) in an area termed nucleus intermedius dorsalis (Ebbesson, 1967).

Discussion

In the present study the presence of spinocerebellar, spinorhombencephalic, spinomesencephalic and spinothalamic projections has been demonstrated in the lizard *Tupinambis nigropunctatus*, the turtle *Testudo hermanni* and the snake *Python reticulatus*. The present findings are readily comparable to the data provided by Ebbesson (1967, 1969). It must be emphasized that so far in reptiles (ten Donkelaar and de Boer - van Huizen, 1978b; Hoogland and Lohman, 1978) only few experimental data are available as regards the cells of origin of the various pathways ascending to the brain stem and diencephalon.

Surveying the experimental data concerning long ascending spinal pathways in terrestrial vertebrates (Fig. 34) the following data should be mentioned:

1) In the frog primary afferent fibers have been traced passing via the dorsal funiculus directly to vestibular nuclei and the cerebellum (Joseph and Whitlock, 1968c). Following dorsal funicular lesions in reptiles also a spinocerebellar pathway via the dorsal funiculus has been shown (Ebbesson, 1967, 1969). However, this pathway is not composed of primary afferent fibers, since the ascending degeneration after dorsal root transections does not extend beyond the dorsal column nuclei.

2) Neither in anurans (Ebbesson, 1969; Hayle, 1973; Wilczynski et al., 1977) nor in urodeles (Nieuwenhuys and Cornelisz, 1971) a direct spinothalamic pathway has been demonstrated: the spinal lemniscus does not extend beyond the mesencephalon. In reptiles, in the pigeon (Karten, 1963) and in mammals, as for instance the opossum (Hazlett et al., 1972) a distinct, although small spino-

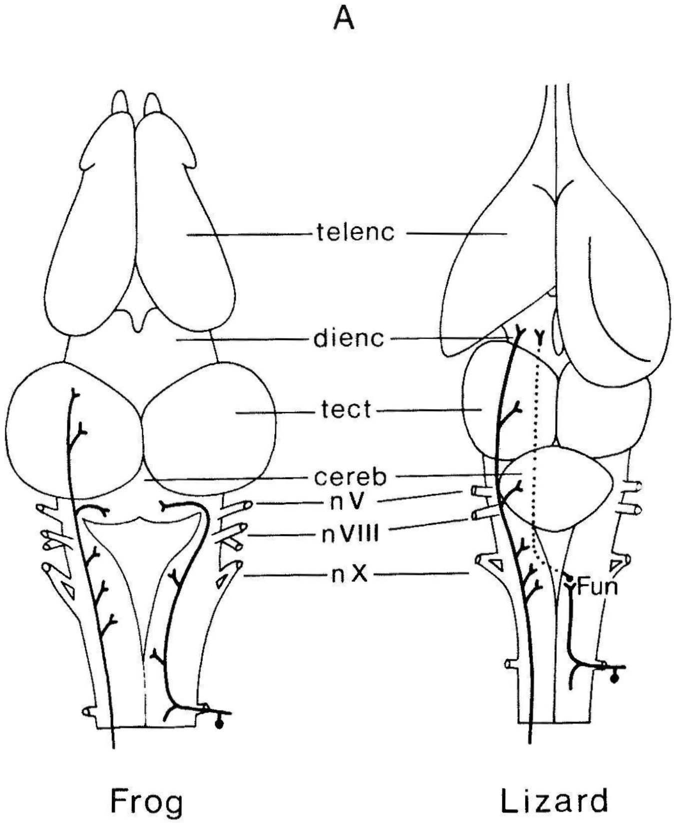


Fig. 34A Semidiagrammatic representation of the primary (at the right) and non-primary (at the left) long ascending spinal pathways demonstrated in the frog and the lizard (after ten Donkelaar and Nieuwenhuys, 1979). The medial lemniscus has also been indicated (dotted line). Abbreviations: cereb, cerebellum; dienc, diencephalon; Fun, nucleus funiculi dorsalis; n V, nervus trigeminus; n VIII, nervus octavus; n X, nervus vagus; tect, tectum mesencephali; telenc, telencephalon.

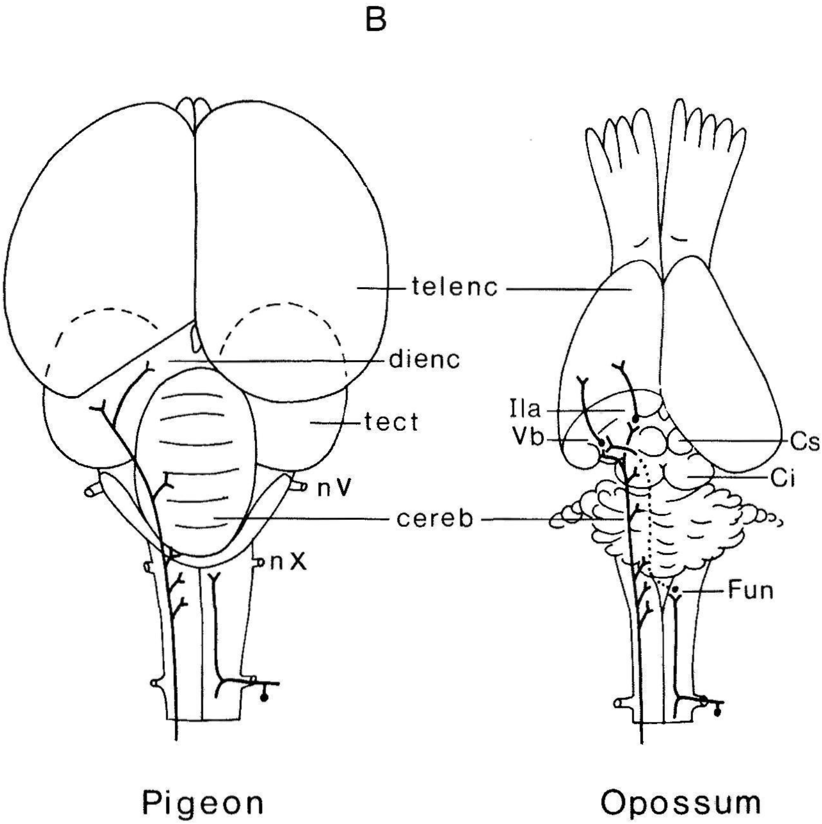


Fig. 34B Semidiagrammatic representation of the primary (at the right) and non-primary (at the left) long ascending spinal pathways demonstrated in the pigeon and the opossum (after ten Donkelaar and Nieuwenhuys, 1979). The medial lemniscus has also been indicated (dotted line). Abbreviations: cereb, cerebellum; Ci, colliculus inferior; Cs, colliculus superior; dienc, diencephalon; Fun, nucleus funiculi dorsalis; Ila, intralaminar nuclei; n V, nervus trigeminus; n X, nervus vagus; tect, tectum mesencephali; telenc, telencephalon; Vb, ventrobasal nuclear complex.

thalamic tract has been found.

3) It should be noted that even in mammals, as for instance the opossum, the spinal lemniscus is composed mainly of spinoreticular fibers. The spinothalamic projection in the opossum can be divided into a paleospinothalamic tract to the intralaminar nuclei and a neospinothalamic tract to the ventrobasal complex, which is a specific sensory relay station. In the opossum the latter tract only accounts for some 2% of the total number of long spinal ascending fibers; in the cat 10%, in higher primates including man 20 - 30% of the spinal lemniscus fibers are neospinothalamic (Mehler, 1969). These quantitative data have been confirmed with the HRP-technique (Trevino and Carstens, 1975): following HRP-injections into the ventrobasal complex labeled cells, i.e. spinothalamic neurons, are more numerous in the monkey's lumbar cord than in the cat's.

4) The spinomesencephalic tract terminates mainly in the nucleus intercollicularis. Evidence for projections from the spinal cord to the intercollicular nucleus has been presented in various vertebrates (cf. RoBards et al., 1976). In various mammals (RoBards et al., 1976) the so-called intercollicular terminal zone receives afferents from the spinal cord, the dorsal column nuclei and the somatosensory cortex. It is concluded that, at least in mammals, this midbrain area might be a major integrative center of the somatosensory system.

5) The presence of a medial lemniscus, i.e. a crossed ascending pathway from the dorsal funicular nuclei, has been definitely shown so far only in mammals (cf. e.g. Hazlett et al., 1972; Hand and van Winkle, 1977) and in the monitor lizard (Ebbesson, 1976b). The medial lemniscus in this reptile appears to terminate in the contralateral ventrolateral thalamic area, the same location where the ventral spinothalamic fibers terminate. In the frog there are indications for the presence of such a pathway (cf. Ebbesson, 1976a; Nieuwenhuys and Opdam, 1976; Wilczynski et al., 1977).

B Descending pathways from the brain stem to the spinal cord

In previous studies (Robinson, 1969; ten Donkelaar, 1976a, b; ten Donkelaar and de Boer - van Huizen, 1978a) the course and site of termination as well as the cells of origin of descending pathways from the brain stem to the spinal cord have been demonstrated in various reptiles. In this chapter some notes on the cells of origin of descending pathways in the lizard *Tupinambis nigropunctatus* and the turtle *Pseudemys scripta elegans* will be presented. Before doing so the results in the previous studies mentioned above will be summarized.

In the three reptiles studied (ten Donkelaar, 1976a, b) the presence of interstitiospinal, vestibulospinal and reticulospinal pathways has been demonstrated. A crossed rubrospinal tract has been shown in the lizard *Tupinambis nigropunctatus* and the turtle *Testudo hermanni*, but could not be demonstrated in the snake *Python reticulatus*. It is safe to assume that reticulospinal fibers, present in the ventral as well as in the lateral funiculus, constitute the bulk of the descending fibers to the spinal cord in reptiles. Reticulospinal fibers in the lateral funiculus arise in the nucleus reticularis inferior and in the nucleus raphes inferior, whereas reticulospinal fibers in the ventral funiculus originate in the nuclei reticulares isthmi, - superior, and - medius (Robinson, 1969; ten Donkelaar, 1976a).

In the lizard *Tupinambis nigropunctatus* and the turtle *Testudo hermanni* a distinct rubrospinal tract has been demonstrated (ten Donkelaar, 1976b) which terminates in the intermediate zone, i.e. in the lateral part of area V - VI, in *Tupinambis* in part also in area IV. The interstitiospinal, vestibulospinal and reticulospinal tracts in *Tupinambis nigropunctatus*, *Testudo hermanni* and *Python reticulatus* all terminate in the medial part of the ventral horn, namely in the medial part of area VII - VIII and the adjacent part of area X (cf. Fig. 35).

It appeared that the classification of descending pathways as advocated in mammals by Kuypers (1964, 1973) into lateral and medial systems can be readily applied to reptiles (ten Donkelaar,

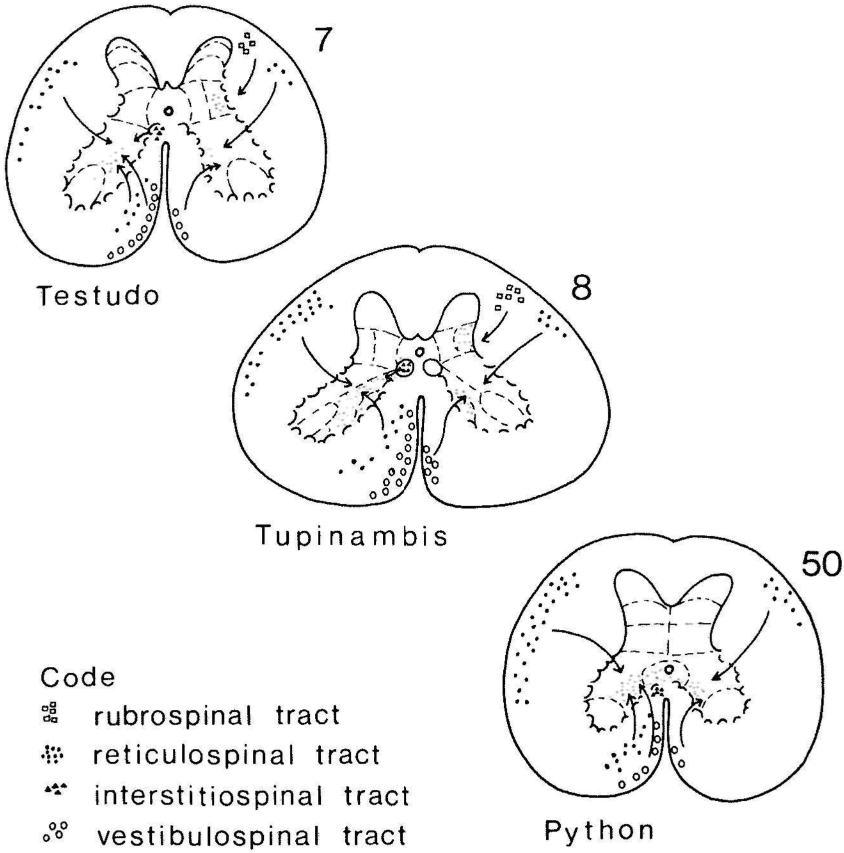


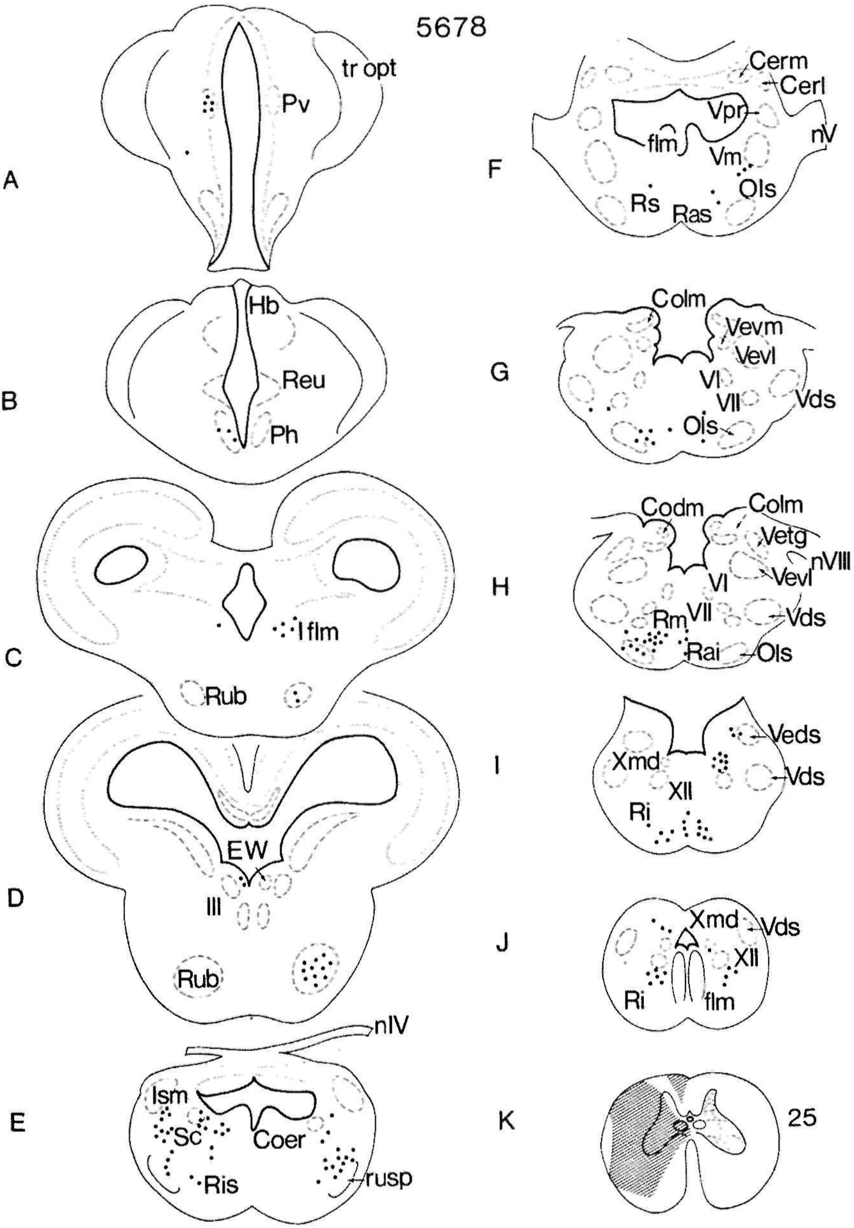
Fig. 35 Schematic diagram summarizing the course and site of termination of the supraspinal descending pathways, represented in approximately comparable transverse sections, viz., the cervical intumescence in the lizard *Tupinambis nigropunctatus* and the turtle *Testudo hermanni*, and the 50th spinal segment in the snake *Python reticulatus*. Small dots represent evidence of (pre)terminal degeneration (modified after ten Donkelaar, 1976b).

1976b). The lateral system terminates in the dorsal and lateral parts of the intermediate zone, the medial system predominantly in the dorsomedial part of the ventral horn. This classification renders it likely that the absence of a rubrospinal tract in *Python reticulatus* is correlated to the absence of limbs.

A comparison of experimental data concerning the systems descending from the brain stem to the spinal cord in amphibians, reptiles, birds and mammals suggests that these systems with regard to origin, course and termination have a basic pattern in common.

The cells of origin of descending pathways to the spinal cord as revealed with the horseradish peroxidase technique will now be discussed. The distribution of the retrogradely labeled neurons after a series of HRP-injections into the 25th spinal segment of the lizard *Tupinambis nigropunctatus* is shown in Figure 36 and will serve as an example. In this experiment the most lateral part of the lateral funiculus and the greater part of the ventral funiculus were spared (Fig. 36K). Retrogradely labeled neurons were present in the diencephalon, in the mesencephalon and throughout the rhombencephalon. In the diencephalon labeled neurons were present in the ipsilateral periventricular hypothalamic nucleus and a few labeled cells were also observed in the ipsilateral paraventricular nucleus (Fig. 36A, B). In the mesencephalon labeled neurons were present in the interstitial nucleus of the fasciculus longitudinalis medialis (Fig. 36C), the ipsilateral nucleus of Edinger-Westphal as well as in the contralateral red nucleus (Fig. 36D). No labeled cells were found in the tectum mesencephali. In the rhombencephalon labeled neurons were present in various parts of the reticular formation (Figs. 36E-J), in the ipsilateral locus coeruleus and the subcoeruleus area (Fig. 36E), and in the contralateral dorsal motor nucleus of the vagal nerve (Fig. 36I). Apart from the labeled neurons in the magnocellular part of the rhombencephalic reticular formation, in addition various small and medium-sized cell groups were observed, e.g. close to the rubrospinal tract (Fig. 36E) and a cell group lying close to the oliva superior (Fig. 36F). No labeled cells were found in the vestibular nuclear complex. It should be noted, however, that a large part of the ventral funiculus, which is known to be occupied by vestibulospinal fibers (ten Donkelaar, 1976b), was not involved by the injections (Fig. 36K).

In the turtle *Pseudemys scripta elegans* (Fig. 37) comparable results were obtained as regards the distribution of labeled cells in the brain stem and diencephalon following HRP-injections into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the 15th spinal segment. In this experiment the injections involved the contralateral dorsal funiculus and a small part of the contralateral ventral funiculus as well (Fig. 37L). Retrogradely labeled neurons in the hypothalamus were present particularly in the ipsilateral periventricular hypothalamic nucleus (Fig. 37B) and one labeled cell was found in the ipsilateral paraventricular nucleus (Fig. 37A). In the mesencephalon labeled cells were present in the interstitial nucleus of the fasciculus longitudinalis medialis (Figs. 37C, D), in the nucleus of Edinger-Westphal and in the contralateral red nucleus (Figs. 37C, D). No labeled cells were found in the tectum mesencephali. In the rhombencephalon labeled neurons were present in various parts of the reticular formation (Figs. 37E-J), in the ipsilateral locus coeruleus (Fig. 37E), in the subcoeruleus area, in the vestibular nuclear complex (Fig. 37G) and in the dorsal motor nucleus of the vagal nerve (Fig. 37J, K). A rostral group of cells, particularly contra-

Fig. 36 The distribution of labeled neurons in the brain stem and diencephalon after HRP-injections into the 25th spinal segment of the lizard *Tupinambis nigropunctatus*. Every labeled cell on 3 consecutive serial sections is plotted on each drawing. Abbreviations: cereb, cerebellum; Cerl, nucleus cerebelli lateralis; Cerm, nucleus cerebelli medialis; Coer, locus coeruleus; Codm, nucleus cochlearis dorsalis magnocellularis; Colm, nucleus cochlearis laminaris; cp, commissura posterior; EW, nucleus of Edinger-Westphal; flm, fasciculus longitudinalis medialis; Hb, habenula; Iflm, nucleus interstitialis of the flm; Ism, nucleus isthmi pars magnocellularis; n IV, nervus trochlearis; n V, nervus trigeminus; n VIII, nervus octavus; Ols, oliva superior; Ph, nucleus periventricularis hypothalami; Pv, nucleus paraventricularis; Rai, nucleus raphes inferior; Ras, nucleus raphes superior; Reu, nucleus reuniens; Ri, nucleus reticularis inferior; Ris, nucleus reticularis isthmi; Rm, nucleus reticularis medius; Rs, nucleus reticularis superior; Rub, nucleus ruber; rusp, tractus rubrospinalis; Sc, subcoeruleus area; tect, tectum mesencephali; tr opt, tractus opticus; Veds, nucleus vestibularis descendens; Vetg, nucleus vestibularis tangentialis; Vevl, nucleus vestibularis ventrolateralis; Vevm, nucleus vestibularis ventromedialis; III, nucleus nervi oculomotorii; Vds, nucleus descendens nervi trigemini; Vm, nucleus motorius nervi trigemini; Vpr, nucleus princeps nervi trigemini; VI, nucleus nervi abducentis; VII, nucleus (motorius) nervi facialis; Xmd, nucleus motorius dorsalis nervi vagi; XII, nucleus nervi hypoglossi.

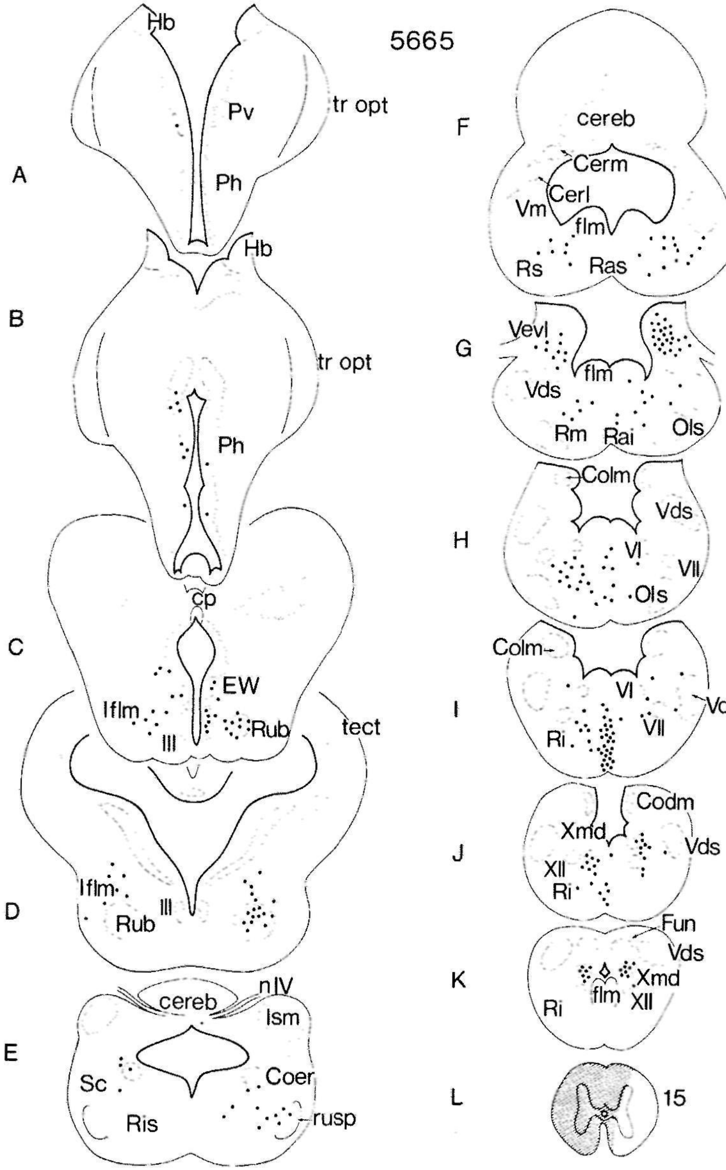

Fig. 37 The distribution of labeled neurons in the brain stem and diencephalon after HRP-injections into the 15th spinal segment of the turtle *Pseudemys scripta elegans*. Each level represents the composite of the plots of 10 sections. For abbreviations cf. Fig. 36.

laterally, lying close to the rubrospinal tract was also found labeled (Fig. 37E).

Discussion

The present findings indicate that in the lizard *Tupinambis nigropunctatus* and the turtle *Pseudemys scripta elegans*, the brain stem and hypothalamus contain a considerable number of cell groups which give rise to descending projections to the spinal cord. The results in these reptiles are readily comparable to data provided in *Lacerta galloti* (ten Donkelaar and de Boer - van Huizen, 1978a). In the lizard *Lacerta galloti* apart from labeled cell groups in the reticular formation and vestibular nuclear complex, the presence of spinal projections of hypothalamic nuclei, somatosensory relay nuclei, deep cerebellar nuclei and two presumably parasympathetic nuclei (nucleus of Edinger-Westphal and dorsal motor nucleus of the vagal nerve) has been demonstrated (ten Donkelaar and de Boer - van Huizen, 1978a).

A comparison of the present findings and those of ten Donkelaar and de Boer - van Huizen (1978a) in *Lacerta galloti* with data in mammals (Castiglioni et al., 1977; Gallaway et al., 1977; Kuypers and Maisky, 1975, 1977; Loewy et al., 1978; Matsushita and Hosoya, 1978; Schoenen and Domesick, 1977) reveals that the pathways descending to the spinal cord in these reptiles show remarkable similarities with pathways in mammals as regards their cells of origin.

X SUMMARY

In this final chapter the main results of the present thesis will be summarized, some variations in the organization of the reptilian spinal cord related to different locomotion patterns will be indicated, and certain general trends in the organization of the spinal cord in terrestrial vertebrates will be surveyed.

1) *Gross structure*: The reptiles studied show profound differences with regard to shape and development of the trunk, tail and extremities. These differences are clearly reflected in the gross structure of the spinal cord. In forms without extremities, like snakes, the cord shows no cervical or lumbar enlargements, but these swellings are well-marked in turtles, lizards and crocodiles, i.e. the occurrence of cervical and lumbar intumescences is correlated with the development of limbs. In the snake *Python reticulatus*, an enlargement of the spinal cord is present, which is definitely related to the well-developed main part of its trunk, and which, hence may be designated as the intumescentia trunci.

2) *Frontal accumulation*: As regards the long ascending fibers it is obvious that these fibers increase in number rostrally, and therefore effect an increase in the cross-sectional area of the white matter. This phenomenon is known as frontal accumulation. It is reported to be clearly evident in reptiles (Ariëns Kappers et al., 1936). However, in the present study such an accumulation has only been observed in the turtle *Testudo hermanni*. The absence of frontal accumulation in the lizard *Tupinambis nigropunctatus* and the snake *Python reticulatus*, is probably due to variations in the number of propriospinal fibers at different levels, which mask the effect of additions from each segment of a few long ascending fibers.

3) *Cytoarchitectonics*: The cytoarchitectonic subdivision of the reptilian spinal cord as presented in this study clearly reflects the influence of Rexed's laminar approach, especially as regards the dorsal horn. The spinal gray has been divided into a number of areas. The neutral term area has been used since not all cell

groups are distinguishable as laminae. It should be stressed once more that while some layers are clear, others are less so, and their delineation is somewhat arbitrary. In general, it may be stated that most boundaries between the areas in the dorsal horn are distinct, whereas the cellular elements of the more ventral areas are more diffusely arranged. In the lizard *Tupinambis nigropunctatus* the motoneuron area consists of two columns, a medial and a lateral one. The former, which is present throughout the spinal cord, is related to the innervation of neck, trunk and tail musculature, whereas the lateral column of motoneurons is present only in the cervical and lumbar enlargements. This lateral column is related to the innervation of the extremity muscles. In the turtle *Testudo hermanni* also two major subdivisions are found, a medial group consisting of motoneurons innervating neck and tail musculature, and a more lateral group, which is present only in the enlargements. A lateral column of motoneurons is absent in the snake *Python reticulatus*.

In terrestrial vertebrates a strong resemblance has been shown to exist in the organization of the spinal gray: it appears that Rexed's parcellation of the spinal gray can be applied to all amniotes discussed in the present investigation.

4) *The distribution of dorsal root fibers into the spinal cord*: At the site of entrance into the spinal cord no clear segregation of large fibers medially and smaller fibers laterally has been observed. A peculiarity for reptiles seems to be a lateral bundle of primary afferent fibers which traverses the dorsal part of the lateral funiculus. The fibers of this bundle enter the spinal gray at the lateral side of the dorsal horn.

Notable variation in dorsal root distribution has been observed in reptiles. In *Testudo hermanni*, *Python reticulatus* and also in *Caiman sclerops* (Joseph and Whitlock, 1968a) almost no fibers were found to extend into the ventral horn. However, in lizards, such as *Tupinambis nigropunctatus*, *Ctenosaura hemilopha* and *Iguana iguana* (Joseph and Whitlock, 1968a), a distinct projection into the ventral horn was observed.

The mode of termination of dorsal root fibers in the various terrestrial vertebrates has been discussed. It seems likely (Joseph and Whitlock, 1968a; Ebbesson, 1976a) that the primary afferent fibers terminate on different parts of the dendritic trees of motoneurons: in frogs and in reptiles like turtles, snakes and caimans primary afferent fibers terminate on distal parts of the dendritic trees of motoneurons. In lizards and in the pigeon more proximal parts of the motoneuronal dendrites are reached, whereas in mammals also axosomatic synaptic contacts have been found.

5) *Propriospinal connections*: The presence of short and long propriospinal fibers has been shown with anterograde degeneration techniques in the lizard *Tupinambis nigropunctatus* and the turtle *Testudo hermanni*. In the snake *Python reticulatus* only short propriospinal fibers could be demonstrated. The presence of cells of origin of short as well as long descending propriospinal pathways has been demonstrated by injecting HRP into the lumbar cord of lizards and turtles. Labeled neurons in the thoracic segments were present particularly in the lateral part of area VII - VIII, predominantly ipsilaterally. Labeled neurons in the cervical intumescence were present in the medial part of area VII - VIII, particularly contralateral to the injection side. The cells of origin of ascending propriospinal pathways are difficult to demonstrate with the present technique, since the HRP-injections also damaged axons of tract cells. It should be noted, however, that following unilateral HRP-injections into the spinal gray of the cervical intumescence of the lizard *Varanus exanthematicus* and the turtle *Pseudemys scripta elegans*, a few labeled neurons were found in the lumbar enlargement in the contralateral area VII - VIII. The long propriospinal fibers demonstrated with anterograde degeneration techniques terminate in the same part of the spinal gray where the cells of origin of such fibers are situated, i.e. the medial part of area VII - VIII.

It seems likely that the organization of the propriospinal connections in reptiles is readily comparable to that in mammals.

Findings in the cat suggest that the propriospinal connections follow the same organizational principles as those governing the descending brain stem pathways (Kuypers, 1973). The bulk of the propriospinal fibers is short and terminates in nearby segments. However, the fibers in the ventral funiculus which are distributed to the ventromedial portion of the intermediate zone (lamina VIII) travel over much longer distances, some of them interconnecting the enlargements. These long propriospinal fibers are mainly derived from neurons situated in lamina VIII (Molenaar and Kuypers, 1978).

The long propriospinal fibers which interconnect the intumescences in quadrupedal reptiles such as *Tupinambis nigropunctatus* and *Testudo hermanni*, are of great importance for the coordination of forelimb and hindlimb movements. It seems likely that the several different ways by which snakes move involve short propriospinal fibers.

6) *Pathways ascending to the brain stem and diencephalon*: In all reptiles studied long ascending pathways to the brain stem and diencephalon have been demonstrated. Primary afferent fibers pass via the dorsal funiculus and terminate in the dorsal funicular nuclei. These long ascending fibers show a gross somatotopical arrangement. Non-primary afferent fibers passing via the superficial zone of the lateral funiculus terminate particularly in the reticular formation and in the cerebellum. In addition, in all reptiles studied a distinct spinomesencephalic projection to the intercollicular nucleus and a small spinothalamic tract have been demonstrated. The presence of a spinothalamic tract has been experimentally shown in all terrestrial vertebrates, except in amphibians.

It must be stressed that so far in reptiles and other nonmammalian vertebrates little is known concerning the cells of origin of the various ascending pathways to the brain stem and diencephalon. However, in a recent study in the lizard *Lacerta galloti* (ten Donkelaar and de Boer - van Huizen, 1978b) it has been shown that the cells of origin of the ascending supraspinal fibers are predominantly situated in the contralateral areas V - VI and VII - VIII.

7) *Pathways descending to the spinal cord*: In the present study the cells of origin of descending pathways from the hypothalamus, mesencephalon and rhombencephalon have been demonstrated in the lizard *Tupinambis nigropunctatus* and the turtle *Pseudemys scripta elegans*. The results in these reptiles are readily comparable to data provided in the lizard *Lacerta galloti* (ten Donkelaar and de Boer - van Huizen, 1978a).

A comparison of the present findings with data in mammals suggests that the pathways descending to the spinal cord in these reptiles show remarkable similarities with pathways in mammals as regards their cells of origin.

8) *Origin and termination of fiber systems in the spinal gray of terrestrial vertebrates*: As regards the origin and termination of fiber systems in the spinal gray of anurans, reptiles, birds and mammals the following remarks can be made:

a. The pattern of distribution of the dorsal root allows a rough lamina by lamina comparison of the dorsal horn in reptiles, birds and mammals.

b. The pathways descending from the brain stem to the spinal cord in terrestrial vertebrates terminate in comparable areas of the spinal gray (ten Donkelaar, 1976b). The lateral system of descending brain stem pathways, i.e. the rubrospinal tract terminates in the lateral part of area V - VI, whereas the medial system composed of interstitiospinal, reticulospinal and vestibulospinal tracts projects predominantly to the ventromedial part of area VII - VIII. A comparison of the present HRP-findings with such data in mammals suggests that the pathways descending to the spinal cord in reptiles, as regards their cells of origin, show remarkable similarities with that in mammals.

c. The long propriospinal fibers in lizards and turtles terminate in the ventromedial part of area VII - VIII, i.e. the area in which the medial system of descending pathways from the brain stem also terminates. Such a coincidence of this termination area has also been observed in the cat (Giovanelli Barilari and Kuypers, 1969). It seems likely that the organization of the propriospinal connections in reptiles is readily comparable to that in mammals (Giovanelli Barilari and Kuypers, 1969; Kuypers, 1973; Molenaar and Kuypers, 1978).

d. The course and site of termination of ascending pathways in the lateral as well as in the dorsal funiculus is very similar to that found in mammals. Spinocerebellar and crossed secondary sensory pathways are present in all terrestrial vertebrates. Since little is known concerning the cells of origin of non-primary ascending pathways in non-mammalian vertebrates, a detailed layer by layer comparison as regards these cells of origin of long ascending spinal pathways must await further connectional data.

Het onderzoek beschreven in dit proefschrift had vooral ten doel meer inzicht te verkrijgen in de bouw en struktuur van het ruggemerg van enkele reptielen. Voor dit onderzoek werden drie species met zeer verschillend lokomotietype gekozen, namelijk de tegu hagedis *Tupinambis nigropunctatus*, die bij de lokomotie gebruik maakt van zowel romp- als extremiteitsspieren, de schildpad *Testudo hermanni*, die zich uitsluitend met behulp van de extremiteiten voortbeweegt, en de slang *Python reticulatus*, die zich uitsluitend met behulp van de rompspieren voortbeweegt. Nagegaan werd in hoeverre deze verschillen in wijze van voortbewegen met verschillen in bouw en struktuur van het centrale zenuwstelsel, in het bijzonder het ruggemerg, samenhangen. Tevens is een poging ondernomen om struktuur en vezelverbindingen van het ruggemerg bij de bestudeerde reptielen te vergelijken met gegevens betrekking hebbend op andere landvertebraten.

Het eerste deel van dit proefschrift omvat een systematische vergelijkende analyse van het cel- en vezelpatroon van het ruggemerg bij reptielen. Deze analyse werd ondernomen omdat voor een exakte beschrijving en duiding van verloop en eindigingswijze van de achterwortelprojekties, van propriospinale verbindingen zowel als van de descenderende systemen vanuit de hersenstam, kennis van het vezelpatroon en de cytoarchitektoniek noodzakelijk is.

Tevens werd experimenteel-anatomisch onderzoek verricht naar het verloop en de eindigingswijze van de vezels die via de achterwortels het ruggemerg binnentreden, en naar de ascenderende zowel als descenderende propriospinale verbindingen. De genoemde vezelsystemen werden bestudeerd met behulp van zilverdegeneratietechnieken (Nauta en Gygax, 1954; Fink en Heimer, 1967). De oorsprongscellen van de propriospinale banen werden met behulp van de horseradish peroxidase (HRP)-tracer techniek bestudeerd. Bij het onderzoek van de propriospinale systemen werd met name gelet op de aanwezigheid van lange propriospinale banen tussen de intumescenties, die van belang zouden kunnen zijn voor de koördinatie van de bewegingen van de extremiteiten (cf. Miller en van der Burg, 1973).

De resultaten van dit onderzoek kunnen als volgt worden samengevat:

1) *Makroskopie*: De extreme verschillen in lichaamsvorm en lokomotietype van de onderzochte reptielen vinden hun weerslag in het centrale zenuwstelsel, met name in het ruggemerg. In vormen die zich uitsluitend met behulp van rompspieren voortbewegen, zoals slangen, vertoont het ruggemerg geen cervicale en lumbale intumescenties. Deze ruggemergsaanzwellingen zijn duidelijk aanwezig in het ruggemerg van de hagedis en de schildpad. Bij de slang is er echter ook een ruggemergsaanzwelling aanwezig, gerelateerd aan het goed ontwikkelde middelste deel van de romp. Deze verdikking werd de intumescentia trunci genoemd.

2) *Frontale accumulatie*: De ascenderende systemen die zich in de witte stof van het ruggemerg bevinden, nemen rostraalwaarts geleidelijk in omvang toe. Dit verschijnsel is bekend als frontale accumulatie. Planimetrisch onderzoek bracht aan het licht dat frontale accumulatie slechts bij de schildpad kan worden waargenomen. De afwezigheid van frontale accumulatie bij de hagedis en de slang hangt waarschijnlijk samen met variaties in het aantal propriospinale vezels op verschillende ruggemergsniveaus die het additie-effekt van de lange ascenderende vezels maskeren.

3) *Cytoarchitektoniek*: Een onderzoek aan de hand van Nissl-materiaal heeft aangetoond dat de grijze stof van het ruggemerg bij deze reptielen in een aantal areas kan worden ingedeeld. Deze indeling vertoont duidelijke overeenkomsten met Rexed's (1952, 1954, 1964) laagsgewijze indeling van de grijze stof van het ruggemerg bij zoogdieren. De neutrale term area werd ingevoerd omdat niet alle celgebieden als lagen zijn waargenomen. Bij de hagedis en de schildpad zijn er twee longitudinale motoneuron kolommen aanwezig, een mediale en een laterale kolom. De laatstgenoemde kolom is slechts aanwezig ter hoogte van de intumescenties. Bij de slang vormen de motoneuronen één enkele kontinue kolom. Grote motoneuronen zijn bij dit dier voornamelijk gelokaliseerd in de voorhoorn van de ruggemergsegmenten ter hoogte van de zogenaamde intumescentia trunci.

4) *Achterwortelprojekties:* Bij de bovengenoemde reptielen werden de projekties van de achterwortels bestudeerd met behulp van:

a. normaal materiaal gekleurd volgens Klüver en Barrera (1953) en Häggqvist (1936),

b. anterograde axondegeneratietechnieken (Nauta en Gygax, 1954; Fink en Heimer, 1967), en

c. HRP-applikatie op de proximale stomp van de doorgesneden achterwortel (een gemodificeerde techniek naar Proshansky en Egger, 1977).

Het onderscheid tussen een dikvezelige mediale en een dunvezelige laterale bundel zoals bij zoogdieren wordt gevonden, is in reptielen niet duidelijk. Allerlei vezeltypen zijn dooreen gemengd aanwezig. De binnenkomende achterwortel splitst zich in een dorsomediale en een ventrolaterale bundel. Deze laatstgenoemde bundel loopt door het dorsale deel van de zijstreng, en treedt uiteindelijk het laterale deel van de grijze stof binnen. De achterwortelprojektie strekt zich bij de hagedis *Tupinambis nigropunctatus* uit tot in het dorsale deel van de laterale motoneuron kolom. Een vergelijkend onderzoek naar de achterwortelprojekties bij de landvertebraten maakt het waarschijnlijk dat de primaire afferente vezels bij deze vertebraten op verschillende delen van de dendrietboom van de motorische voorhoorn cellen eindigen. Bij kikkers en bij reptielen als schildpadden, slangen en krokodillen, eindigen de primaire afferente vezels op de distale delen van de dendrietboom. Bij hagedissen en bij de duif bereiken deze vezels de meer proximale delen van de dendrietboom, terwijl bij zoogdieren ook axosomatische synapsen zijn waargenomen.

5) *Propriospinale verbindingen*: De aanwezigheid van zowel korte als lange propriospinale vezels werd aangetoond bij de hagedis *Tupinambis nigropunctatus* en bij de schildpad *Testudo hermanni*. Bij de slang *Python reticulatus* werden slechts korte propriospinale vezels gevonden. De oorsprongscellen van korte en lange descenderende propriospinale vezels werden bestudeerd met behulp van HRP-injekties in het lumbale ruggemerg van hagedissen en schildpadden. Retrograad gelabelde cellen in de thoracale segmenten bleken voornamelijk

gelokaliseerd te zijn in het laterale deel van de ipsilaterale area VII - VIII. In de cervicale intumescentie waren de gelabelde cellen gelokaliseerd in het mediale deel van area VII - VIII, voornamelijk kontralateraal ten opzichte van de injektieplaats. De oorsprongscellen van ascenderende propriospinale banen zijn met deze techniek moeilijk te onderscheiden van die van lange ascenderende vezels van het ruggemerg naar de hersenstam. Opmerkelijk is dat er na ëënzijdige HRP-injekties in de grijze stof van de cervicale intumescentie bij de varaan *Varanus exanthematicus* en bij de schildpad *Pseudemys scripta elegans*, enkele gelabelde cellen in de kontralaterale area VII - VIII van de lumbale intumescentie werden waargenomen.

Het lijkt waarschijnlijk dat de organisatie van de propriospinale verbindingen bij reptielen goed vergelijkbaar is met die bij zoogdieren (Giovanelli Barilari en Kuypers, 1969; Kuypers, 1973; Molenaar en Kuypers, 1978).

Lange propriospinale vezels die de ruggemergsaanzwellingen van viervoetige reptielen zoals *Tupinambis nigropunctatus* en *Testudo hermanni*, met elkaar verbinden, lijken van bijzonder belang voor de bewegingskoördinatie van de voor- en achterpoten.

6) *Banen opstijgend naar hersenstam en diencephalon*: Lange ascenderende banen naar hersenstam en diencephalon werden bij alle drie bestudeerde reptielen aangetoond. De primaire afferente vezels treden de achterstreng binnen en stijgen daarin op tot aan de achterstrengkernen. Deze lange ascenderende vezels in de achterstreng vertonen een somatotopische ordening in die zin dat vezels van kaudale oorsprong meer mediaal gesitueerd zijn, en de zich rostraal bijvoegende vezels meer lateraal komen te liggen. Niet-primaire afferente vezels lopen in het oppervlakkige deel van de zijstreng en eindigen grotendeels in de reticulaire formatie en in het cerebellum. Tevens werden een duidelijke spinomesencephale baan die naar de nucleus intercollicularis projekteert en een kleine spinothalamische baan aangetoond. De aanwezigheid van een spinothalamische verbinding is ook experimenteel aangetoond bij andere landvertebraten, met uitzondering van amfibieën.

7) *Descenderende banen naar het ruggemerg*: In dit proefschrift werden met behulp van de HRP-tracer techniek de oorsprongscellen van de naar het ruggemerg afdalende banen bestudeerd bij de hagedis *Tupinambis nigropunctatus* en de schildpad *Pseudemys scripta elegans*. Descenderende verbindingen vanuit de hypothalamus en vanuit verschillende gebieden in het mesencephalon en het rhombencephalon werden aangetoond. De resultaten bij deze reptielen zijn direkt vergelijkbaar met die bij de hagedis *Lacerta galloti* (ten Donkelaar en de Boer - van Huizen, 1978a).

Een vergelijking van deze gegevens bij reptielen met die bij zoogdieren suggereert dat de descenderende verbindingen vanuit de hersenstam en het diencephalon naar het ruggemerg, in het bijzonder wat betreft hun oorsprongscellen, in grote lijnen vergelijkbaar zijn.

8) *Oorsprong en eindigingswijze van de vezelsystemen in de grijze stof van verschillende vertebraten*: Een vergelijking betreffende oorsprong en eindigingswijze van de vezelsystemen in de grijze stof bij amfibieën, reptielen, vogels en zoogdieren geeft aanleiding tot de volgende opmerkingen.

a. Het distributiepatroon van de achterwortelvezels in de achterhoorn bij reptielen, vogels en zoogdieren is in grote lijnen vergelijkbaar.

b. De naar het ruggemerg afdalende banen bij verschillende vertebraten eindigen in de grijze stof in vergelijkbare areas (ten Donkelaar, 1976b). Het laterale systeem van de descenderende hersenstam banen, te weten de rubrospinale baan, eindigt in het laterale deel van area V - VI, terwijl het mediale systeem bestaande uit interstitiospinale, reticulospinale en vestibulospinale banen, voornamelijk projekteert naar het ventromediale deel van area VII - VIII. Een vergelijking van de in dit onderzoek verkregen HRP resultaten met gegevens bij zoogdieren suggereert dat de naar het ruggemerg afdalende banen, wat betreft hun oorsprongscellen, vergelijkbaar zijn.

c. Lange propriospinale vezels bij hagedissen en schildpadden eindigen in het ventromediale deel van area VII - VIII, dat is het gebied waarin het mediale systeem van descenderende hersenstam banen ook eindigt. Vergelijkbare gegevens werden ook bij de rat verkregen (Giovanelli Barilari en Kuypers, 1969). Waarschijnlijk is de organisatie van de propriospinale verbindingen bij reptielen vergelijkbaar met die bij zoogdieren (Giovanelli Barilari en Kuypers, 1969; Kuypers, 1973; Molenaar en Kuypers, 1978).

d. De vezelsystemen die in de zijstreng en in de achterstreng opstijgen zijn, zowel wat hun verloop als hun eindigingswijze betreft, goed met de corresponderende systemen bij zoogdieren vergelijkbaar.

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CURRICULUM VITAE

Arinardi Kusuma werd geboren op 14 mei 1946 te Malang, Indonesië. Hij bezocht de Algemene Middelbare School "St. Albertus" aldaar en behaalde in 1964 het einddiploma SMA-B (Wis- en Natuurkundige afdeling). Aansluitend begon hij zijn studie in de geneeskunde aan de voormalige Medische Hogeschool te Malang (STKM), waar hij tot 1970 de doctoraalopleiding volgde. Van 1966 tot 1970 was hij tevens werkzaam als assistent op het Laboratorium voor Anatomie van de bovengenoemde Medische Hogeschool.

Daar het destijds voor hem niet mogelijk was het doctoraalexamen geneeskunde in Indonesië af te leggen, vertrok hij in 1970 naar Nederland. In 1971 vervolgde hij zijn studie aan de Katholieke Universiteit te Nijmegen en in 1974 werd het doctoraalexamen in de Vrije Studierichting Geneeskunde afgelegd. Vanaf 1972 is hij werkzaam op het Instituut voor Anatomie en Embryologie (Hoofd: Prof. Dr. H.J. Lammers) van de Fakulteit der Geneeskunde en Tandheelkunde aan de Katholieke Universiteit te Nijmegen, aanvankelijk als student-assistent en vanaf januari 1975 als wetenschappelijk medewerker.

Vanaf november 1974 zette hij de studie gericht op het behalen van het artsdiploma voort. In oktober 1976 werd het doctoraalexamen geneeskunde en in juni 1978 het semi-artsexamen afgelegd.

STELLINGEN

I

Het ruggemerg is veruit het grootste, maar ook veruit het minst onderzochte deel van het centrale zenuwstelsel van reptielen.

II

De recentelijk ontwikkelde tracer-technieken (retrograad transport van het enzym mierikswortel peroxydase, anterograad transport van radioaktief gemerkte aminozuren), fysiologische technieken (b.v. antidrome stimulatie) zowel als de intracellulaire kleuringstechnieken zijn veelbelovende hulpmiddelen bij het oplossen van problemen met betrekking tot de organisatie van het ruggemerg van de reptielen.

III

Er zijn duidelijke aanwijzingen voor het bestaan van een 'locomotor pattern generator' in het ruggemerg die in gang gezet kan worden door descenderende verbindingen vanuit de hersenstam.

S. GRILLNER (1975): Physiol. Rev. *55*: 247-304
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S. MILLER and P.D. SCOTT (1977): Exp. Brain Res. *30*: 387-403

IV

Grondige kennis van de morfologie van het centrale zenuwstelsel is vereist voor de interpretatie van de CAT-scan en andere vormen van neuroradiodiagnostiek.

V

Een lumbaalpunctie ter verificatie van een compressio medullae kan een 'dreigende' dwarslesie in een complete dwarslesie veranderen.

E. MEIJER (1977): Proefschrift, Nijmegen

VI

Het dogma dat de pyramidebaan (tractus corticospinalis) in de voorste helft van het crus posterius van de capsula interna gelokaliseerd is, is aanvechtbaar.

J. HANAWAY and R.R. YOUNG (1977): J. Neur. Sci. *34*: 63-70

VII

Er komen 'benigne' aandoeningen voor, welke tot 'maligne' stoornissen aanleiding kunnen geven.

R.E. IVERSON and L.M. VISTNES (1973): Am. J. Surg. *126*: 359-365

W. LUYENDIJK (1973): Oncologie (A. ZWAVELING en R.J. VAN ZONNEVELD, red.), pp. 235-242

VIII

Niet alleen psychische symptomen bij organische processen komen veel voor, doch ook somatische klachten bij psychiatrische aandoeningen.

IX

De hik, die niet veroorzaakt wordt of ontstaan is door somatische afwijkingen, kan effektief bestreden worden door de adem zo lang mogelijk in te houden.

X

'Hooikoorts' heeft weinig met hooi te maken en koorts komt er vrijwel nooit bij voor.

A. KUSUMA

Nijmegen, 17 mei 1979